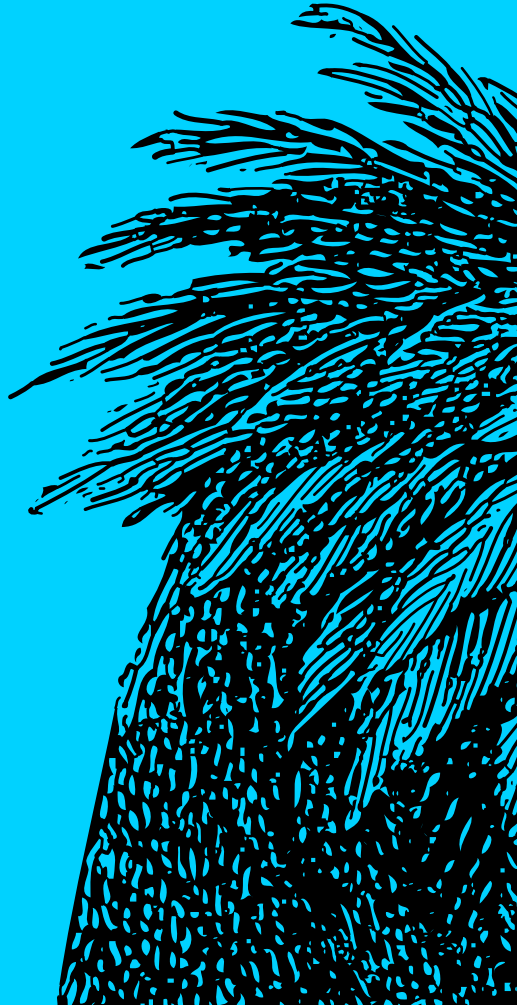

ANIMAL BIOLOGY ABSTRACTS





A1 PHYSIOLOGICAL MECHANISMS OF AQUATIC TOXICOLOGY

ORGANISED BY: TAMZIN BLEWETT (UNIVERSITY OF ALBERTA, CANADA)
AND GREG GOSS (UNIVERSITY OF ALBERTA, CANADA)

A1.1 QUANTITATION AND CAUSALITY OF PROTEOME DYNAMICS IN FISH EXPOSED TO ENVIRONMENTAL STRESS

📅 MONDAY 3 JULY, 2017 ⌚ 09:00

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Fish have evolved to thrive in highly diverse aquatic habitats, including extreme environments that are characterized by large fluctuations of salinity, temperature, and pH. Species inhabiting such stressful environments are considered eury-tolerant because they are capable of utilizing a conserved set of stress response mechanisms for acclimation and adaptation to environmental challenges. Many of these responses are also utilized when fish encounter novel types of stressors such as anthropogenic pollutants and toxicants. To better understand the biological mechanisms associated with such complex stress responses, analyses that provide molecular resolution of dynamic proteomes at system-wide scales are required. Proteins are the functional and structural workhorses of all cells, tissues, and organisms. They represent the targets of almost all pharmaceutical drugs. The abundances, covalent modification states and cell/tissue compartmentation of proteins are highly dynamic and context-dependent. Therefore, proteins are excellent indicators of the biological condition of cells, tissues, and organisms. Recent advances in biological mass spectrometry and computer science have enabled quantification of thousands of specific proteins simultaneously at accuracy and reproducibility superior to immunoblotting approaches. This presentation outlines the construction and use of hyperreaction monitoring (HRM) mass spectrometry assay libraries for quantitation of proteome dynamics during stress acclimation and adaptation of eury-tolerant fish. Data on three spine sticklebacks inhabiting highly diverse environments illustrate how HRM proteomics empowers protein regulatory network construction. Furthermore, HRM proteomics is ideal for scoring molecular phenotypes caused by CRISPR genome editing of stress response genes.

A1.2 POLYSTYRENE NANOBEADS ENHANCE POLYCYCLIC AROMATIC HYDROCARBON GENOTOXICITY IN AN IN VITRO FISH MODEL

📅 MONDAY 3 JULY, 2017 ⌚ 09:40

👤 NICOLAS BURY (UNIVERSITY OF SUFFOLK, UNITED KINGDOM), DANIEL BUSSOLARO (UNIVERSIDADE FEDERAL DO PARANA, BRAZIL), STEPHANIE WRIGHT (KING'S COLLEGE LONDON, UNITED KINGDOM), KRISTIN SCHIRMER (EAWAG, SWITZERLAND), VOLKER ARLT (KING'S COLLEGE LONDON, UNITED KINGDOM)

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Microscopic plastic particles - microplastics - are a ubiquitous contaminant in aquatic environments, which potentially exist at the nanoscale. They bind hydrophobic chemicals, such as polycyclic aromatic hydrocarbons (PAHs), altering their environmental fate, as well as interactions with biota. Using rainbow trout intestine (RTgutGC) and gill (RTgill-W1) cell lines, the present study investigates the effects of polystyrene micro-(MB) (4 µm) and nanobeads (NB) (0.22 µm) on the cyto- and genotoxicity of two PAHs, benzo[a]pyrene (BaP) and 3-nitrobenzanthrone (3-NBA) over 48 hrs. Using the Alamar Blue bioassay to assess cytotoxicity, PAHs (0.1, 1 and 10 µM) decreased cell viability by 10-20% at 10 µM in both cell lines, whereas MBs and NBs (1,000-10,000 particles/ml) were non-toxic. Genotoxicity was assessed using the Comet assay and a measure of oxidative DNA damage via the formamidopyrimidine DNA-glycosylase (FPG)-modified Comet assay. In the gill cell line only minor increases (1-4%) in DNA damage were seen in all treatments. The gut cell line DNA damage increased to 8% for BaP and 15% for 3-NBA, with a greater induction of oxidative DNA damage. The most dramatic result was in the presence of both 0.22 µm NBs and 10 µM 3-NBA, with oxidative DNA damage reaching 69%. This was not observed for the 4 µm MBs. Results show the presence of plastic NBs enhances genotoxicity of 3-NBA, causing a significant increase in DNA damage in the intestine cell line. Dietary exposure to this mixture of contaminants may be of greater concern than respiratory pathways in aquatic species.

A1.3 MECHANISMS OF RESPONSE TO ESTROGENIC ENDOCRINE DISRUPTORS DIFFER IN THE MODEL FISH, THE ESTUARINE KILLIFISH *FUNDULUS HETEROCLITUS*

📅 MONDAY 3 JULY, 2017 ⌚ 09:55

👤 DEBORAH MACLATCHY (WILFRID LAURIER UNIVERSITY, CANADA), THIVIYA KANAGASABESAN (WILFRID LAURIER UNIVERSITY, CANADA), TAMZIN BLEWETT (MCMaster UNIVERSITY, CANADA), ESTEBAN GILLIO MEINA (WILFRID LAURIER UNIVERSITY, CANADA), IBRAHIM CHEHADE (WILFRID LAURIER UNIVERSITY, CANADA), THIJS BOSKER (UNIVERSITY OF NEW BRUNSWICK, CANADA), ANDREA LISTER (WILFRID LAURIER UNIVERSITY, CANADA)

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Different reproductive responses to the environmental estrogen 17 α -ethinylestradiol (EE₂) have been observed across common model teleosts. In the freshwater zebrafish (*Danio rerio*) and fathead minnow (*Pimephales promelas*) exposure of adults to low levels of EE₂ (less than 25 ng/L) causes significant reductions in the number of eggs spawned and plasma steroid levels. However, EE₂ exposure for three weeks at 10-fold higher concentrations does not inhibit spawning in an estuarine killifish, the mummichog (*Fundulus heteroclitus*); mummichog plasma steroids also decrease to a lesser degree during EE₂ exposure. Despite differences in plasma steroids and spawning levels, EE₂ consistently induces vitellogenin in males and gonadal sex reversal in embryos of all three species. Environmental salinity has been eliminated as a factor in uptake and effects of EE₂ in mummichog. EE₂ accumulates differentially (and primarily in liver and gall bladder) in mummichog compared with other common model teleosts indicating one potential key physiological difference (i.e., clearance). Gonadal gene expression of luteinizing hormone receptor (LHr) has been proposed as a robust estrogen-responsive endpoint in fish; however, in killifish, in vitro exposure of maturing oocytes to 0–250 nM of EE₂ for 24 h showed no difference in expression of LHr. Investigation of gene expression throughout the oocyte maturation cycle of selected steroidogenic enzymes and gonadotropin and estrogen receptors demonstrated differential patterns exist between killifish and other studied fish species. Further understanding of gonadal physiology in mummichog is key to determining why responses at the level of egg production and plasma steroids vary.

A1.4 MANGROVES AS BIOFILTERS: THE OTHER SIDE OF THE COIN WITH MANGROVE CRABS BEING DIFFERENTIALLY AFFECTED BY WASTEWATER RELEASE

📅 MONDAY 3 JULY, 2017 ⌚ 10:25

👤 DIMITRI THEUERKAUFF (UNIVERSITY OF MONTPELLIER, FRANCE), JEHAN-HERVÉ LIGNOT (UNIVERSITY OF MONTPELLIER, FRANCE)

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Mangroves forest are increasingly used as bioremediation tool for wastewater (WW) treatment in tropical countries. If mangrove trees benefit from this nutrient load, the impact on the associated macrofauna is unclear. In Mayotte (Comoros archipelago), a pilot study started in 2007 involving the discharge of domestic effluent following primary treatment.

We investigated the effects of this WW on physiological parameters of three key engineering mangrove crabs: *Uca urvillei*, *Neosarmatium meinerti* and *Perisesarma guttatum*. WW consists of a cocktail of contaminants (containing notably high ammonia levels) and could induce a salinity stress (seawater to freshwater). The O₂ consumption rate of submerged and emerged crabs and their osmoregulatory response were investigated. For *U. urvillei*, the effect of WW exposure on the redox metabolism (antioxidant content and reactive oxygen and nitrogen species formation) was also evaluated.

Results showed that *U. urvillei* is physiologically tuned for low salinity but exposure to 0 ppt induces high mortality. *N. meinerti* and *P. guttatum* are stronger hyper/hypo osmoregulators. WW exposure induces a burst in O₂ consumption rate for *N. meinerti* and *U. urvillei* but a metabolic depression for *P. guttatum* that breathes normally in air after acute exposure. WW also induces ROS production in *U. urvillei* but does not impair osmoregulatory capacity compared to low salinity. However, for *N. meinerti*, the osmoregulatory capacity is affected by WW exposure.

This differential vulnerability towards WW among mangrove crab species must be considered in order to provide adequate conservation measures when mangroves are used as biofiltering system.

A1.5 EXAMINING THE EFFECTS OF SHORT-TERM ATRAZINE EXPOSURE ON A NON-TARGET SPECIES, THE RAINBOW DARTER (*ETHEOSTOMA CAERULEUM*)

MONDAY 3 JULY, 2017 10:40

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The herbicide atrazine has been shown to have adverse effects on non-target species, including feminization in males. Rainbow darters (*Etheostoma caeruleum*) are a pollutant sensitive species of freshwater fish commonly found in the Kentucky and Ohio River watersheds of the Midwestern US, where agricultural atrazine use is common. Following seven day exposures to 0, 0.1, 3, and 10 ppb atrazine, potential endocrine disruption in rainbow darters was examined via analysis of: gonad-somatic index (GSI) and liver-somatic index (LSI); gonadal concentrations of estradiol (E2) and testosterone (T); hepatic expression of vitellogenin (VTG); and gonadal expression of aromatase (CYP19a). Results indicate statistical comparisons to controls.

A1.6 BUILDING THE FISH INTESTINE IN VITRO – STUDIES ON BARRIER FUNCTION WITH THE RAINBOW TROUT CELL LINE, RTgutGC

MONDAY 3 JULY, 2017 14:00

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The intestine of fish is a multifunctional organ: lined by only a single layer of specialized epithelial cells, it has various physiological roles including nutrient absorption and ion regulation. It moreover comprises an important barrier for environmental toxicants. Thus far, knowledge of the fish intestine is limited largely to in vivo or ex vivo investigations. Recently, however, a fish intestinal cell line, RTgutGC, was established, originating from a rainbow trout (*Oncorhynchus mykiss*). In order to exploit the opportunities arising from RTgutGC cells for exploring fish intestinal physiology and toxicology, we established culture of these cells on commercially available, as well as on newly designed, ultra-thin aluminium membranes. These membranes serve as support in order to build a two-compartment system in which one compartment represents the gut lumen and the other the interior, blood-facing side. Within three weeks of culture, RTgutGC cells show epithelial features by forming tight junctions and desmosomes between adjacent cells. Cells develop a transepithelial electrical resistance comparable to in vivo measured values, reflecting the leaky nature of the fish intestine. Immunocytochemistry reveals evidence of polarization, such as basolateral localization of Na^+/K^+ -ATPase and apical localization of

the tight junction protein ZO-1. On this background, we work toward different applications of this system including a) the assessment of molecular responses underlying saltwater adaptation; b) the quantification of permeation of chemicals and particles across the intestinal layer as proxy for intestinal uptake and c) non-invasive monitoring of the viability of the cells on aluminium membranes based on impedance spectroscopy.

A1.7 EFFECTS OF CRUDE OIL EXPOSURE ON THE PELAGIC MAHI-MAHI (*CORYPHAENA HIPPURUS*), FROM MOLECULAR ENDPOINTS THROUGH HABITAT UTILIZATION OF WILD FISH

MONDAY 3 JULY, 2017 14:40

MARTIN GROSELL (RSMAS UNIVERSITY OF MIAMI, UNITED STATES), ELVIS GENBO (UNIVERSITY OF CALIFORNIA RIVERSIDE, UNITED STATES), RACHAEL HEUER (RSMAS UNIVERSITY OF MIAMI, UNITED STATES), GEORGINA COX (RSMAS UNIVERSITY OF MIAMI, UNITED STATES), JOHN STIEGLITZ (RSMAS UNIVERSITY OF MIAMI, UNITED STATES), DEREK NELSON (UNIVERSITY OF NORTH TEXAS, UNITED STATES), LELA SCHLENKER (RSMAS UNIVERSITY OF MIAMI, UNITED STATES), DAN SCHLENK (UNIVERSITY OF CALIFORNIA RIVERSIDE, UNITED STATES), DANE CROSSLEY (UNIVERSITY OF NORTH TEXAS, UNITED STATES), DAN BENETTI (RSMAS UNIVERSITY OF MIAMI, UNITED STATES)

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Crude oil is toxic to cardiac development in early life stages of freshwater and marine teleosts. However, more recently, later life stages have also been shown to be highly sensitive to crude oil exposure. RNAseq experiments revealed, among other targets, that cardiac calcium signaling was impacted during oil exposure. Subsequent experiments on isolated ventricular myocytes from mahi showed substantially reduced sarcomere shortening, effects that appear more pronounced at higher stimulation frequencies. Reduced sarcomere shortening is congruent with a reduction in cardiac output noted in oil-exposed adult mahi. These cell and organ level findings ultimately explains impairments to performance in intact animals, including reduced maximal oxygen uptake, aerobic scope, and U_{crit} in adult mahi exposed for 24 hours to $\sim 10 \mu\text{g} \Sigma\text{PAH l}^{-1}$. Reduction in these performance metrics could be catastrophic for mahi given their life history strategies. They have some of the highest growth and reproductive output rates of any marine fish, have the highest in situ cardiac power output of any fish measured to date ($13 \text{ mW g}^{-1} \text{ Mv}$) and among the highest absolute sustained swim speeds (130 cm s^{-1}), rivaled only by certain salmonids. Pop-up satellite tagged mahi in the south Atlantic display extensive diurnal vertical movements, long distance migrations of $\sim 100 \text{ km day}^{-1}$, and span a wide temperature range ($\Delta 10^\circ\text{C}$). Thus, impacts of oil exposure on cardiac performance is likely to alter habitat utilization, migrations, spawning, and survival of wild mahi. This research was made possible by a grant from GoMRI (Grant No: SA-1520).

A1.8 CARDIORESPIRATORY AND METABOLIC PERFORMANCE IMPAIRMENTS IN ZEBRAFISH (*DANIO RERIO*) FOLLOWING ACUTE HYDRAULIC FRACTURING FLOWBACK AND PRODUCED WATER EXPOSURE

📅 MONDAY 3 JULY, 2017 ⌚ 14:55

👤 ERIK J FOLKERTS (UNIVERSITY OF ALBERTA, CANADA), TAMZIN A BLEWETT (UNIVERSITY OF ALBERTA, CANADA), YUHE HE (UNIVERSITY OF ALBERTA, CANADA), GREG G GOSS (UNIVERSITY OF ALBERTA, CANADA)

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Flowback and produced water (FPW) from horizontal hydraulic fracturing activities is an emerging toxicological concern in North America. Although salinity dominates much of the toxicity observed, other inorganic (e.g. metals, radionuclides, etc.) and organic (e.g. polyaromatic hydrocarbons) contaminants found in FPW can affect multiple physiological mechanisms and induce an array of toxicological responses in exposed organisms. The current study analyzing acute embryonic zebrafish (*Danio rerio*) FPW exposures investigated cardiotoxic responses and altered metabolic performance. Zebrafish embryos (24 hpf) were acutely exposed to 2.5% and 5% FPW solutions for 24 or 48 hrs. Developmental deformities analyzed at 120 hpf determined that 2.5%, 48 hr FPW exposures significantly increased pericardial edema, yolk-sac edema, and tail/spine curvatures. Embryonic basal metabolic rates measured at 48, 72, 96, and 120 hpf were significantly reduced following all FPW exposures. To link lowered embryonic metabolic rates after FPW exposure to cardiovascular functioning, qPCR analysis of cardiomyocyte electrogenic and developmental genes, including *atp2a2a* and *nkx2.5*, revealed significantly altered embryonic transcript expression following FPW exposure. Finally, to determine if these embryonic FPW induced sub-lethal effects persisted to later life stages, swim performance (U_{crit} measured in body lengths/s) and swimming metabolic capacity (factorial aerobic scope; F-AS) of juvenile zebrafish (60 dpf exposed as embryos for 24 or 48 h) was analyzed. FPW exposure significantly decreased both juvenile fish U_{crit} and F-AS values. Our study confirms the cardiovascular system as an endpoint for FPW toxicity and validates metabolic impairment as a potential toxicological consequence of FPW exposure.

A1.9 IMPACTS OF OIL EXPOSURE ON MAHI-MAHI (*CORYPHAENA HIPPURUS*) EMBRYOS – METABOLIC COSTS AND BUOYANCY CONTROL

📅 MONDAY 3 JULY, 2017 ⌚ 15:10

👤 CHRISTINA PASPARAKIS (ROSENSTIEL SCHOOL OF MARINE AND ATMOSPHERIC SCIENCE, UNITED STATES), LAUREN E SWEET (UNIVERSITY OF NORTH TEXAS, UNITED STATES), JOHN D STIEGLITZ (ROSENSTIEL SCHOOL OF MARINE AND ATMOSPHERIC SCIENCE, UNITED STATES), DANIEL BENETTI (ROSENSTIEL SCHOOL OF MARINE AND ATMOSPHERIC SCIENCE, UNITED STATES), CONRAD T CASENTE (ROSENSTIEL SCHOOL OF MARINE AND ATMOSPHERIC SCIENCE, UNITED STATES), AARON P ROBERTS (ROSENSTIEL SCHOOL OF MARINE AND ATMOSPHERIC SCIENCE, UNITED STATES), MARTIN GROSELL (ROSENSTIEL SCHOOL OF MARINE AND ATMOSPHERIC SCIENCE, UNITED STATES)

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The Deepwater Horizon oil spill coincided with the spawning window of many ecologically and economically important fish species. Aside from the acute mortality elicited by this event, additional sublethal effects may have subtle yet ecologically significant consequences on populations of pelagic fishes as a whole. In particular, the control of embryonic buoyancy is critical to early survival, as it aids in growth and dispersal by positioning newly hatched larvae in optimal prey-predator fields and facilitating dispersal through ocean currents. We found that co-exposure to environmentally relevant stressors such as high temperature and UV-radiation, in concert with oil, affect the induction and duration of negative buoyancy in mahi embryos. In addition to the early onset of negative buoyancy, oil- and UV-exposed embryos had elevated rates of oxygen consumption (~10 fold) and increased yolk sac depletion compared to control embryos at the same developmental stage. The deleterious effects of oil- and UV-exposure were enhanced at high temperature, suggesting these stressors interact in a way to interfere with hatch, survival and dispersal in pelagic species. The mechanisms behind embryonic buoyancy control are not fully understood, but our findings suggest that regulation of ammonia excretion may fine-tune specific gravity. Responses to UV radiation demonstrate that buoyancy control in pelagic fish embryos may be much more dynamic than previously thought. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).

A1.14 HOW CAN STUDIES OF PHARMACEUTICALS IN THE ENVIRONMENT SUPPORT BASIC AND APPLIED PHYSIOLOGY AND TOXICOLOGY RESEARCH?

📅 MONDAY 3 JULY, 2017 ⌚ 16:10

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The face of the planet is changing with more people now living in cities than ever before. Concentrating people also concentrates resource consumption, including chemical use. When we started studying urban waters and associated contaminants of emerging concern, very little was known about the bioaccumulation and toxicology of human pharmaceuticals in the environment. Fortunately, pharmacology and toxicology information is more readily available for human pharmaceuticals than other classes of environmental contaminants, and numerous pharmacological targets appear functionally conserved across animals and plants. In addition, some physiologists had previously employed pharmaceuticals as positive or negative controls in basic experimental studies with plants and animals. We have focused our efforts toward comparatively understanding bioaccumulation and aquatic toxicology pathways associated with physico-chemical and biological properties of pharmaceutical classes, which routinely differ from many historically studied environmental organic contaminants (e.g., PCBs). In fact, we identified how many pharmaceuticals are not appropriately examined by historically developed predictive models and standardized toxicity testing designs. Here we examine how pharmaceuticals provide useful model substances to test basic comparative hypotheses and to advance an understanding of diverse physiological mechanisms in aquatic toxicology.

A1.15 THE EFFECT OF COPPER NANOPARTICLES ON OLFACTION IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

📅 MONDAY 3 JULY, 2017 ⌚ 16:40

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Fish rely on olfaction for their survival, growth, and reproduction. Metal contaminants (e.g. copper) can impair fish olfaction. Although copper ion (Cu^{2+}) has drawn the most attention in olfactory toxicology studies, the impact of copper nanoparticles (CuNPs) on fish olfactory systems has not been well determined. To our knowledge, there is only one study that has investigated toxicity of CuNPs in fish olfactory-mediated behaviours, but neurophysiological alteration of olfactory sensory neurons has not yet been examined. The objective of this study was to investigate time-dependent effects of CuNPs and Cu^{2+} on olfactory acuity of rainbow trout using electro-olfactography (EOG). To establish CuNPs or Cu^{2+} induced

olfactory-impairment thresholds, inhibitory concentration (IC) curves were determined. Fish were exposed to a geometric dilution series of contaminants (CuNPs or Cu^{2+}) for 24 hours, and fish olfactory acuity was measured using EOG. Afterwards, fish were exposed to CuNPs or Cu^{2+} at concentrations known to impair the olfactory system by 50% (322 and 6.8 $\mu\text{g}/\text{L}$ for CuNPs and Cu^{2+} respectively) for a 24 h or 96 h exposure period. Results revealed that while a 96h exposure to CuNPs causes a significantly greater impairment of fish olfactory function relative to 24h, fish olfactory acuity partially recovered after 96h under continuous Cu^{2+} exposure. Over the same exposure periods, CuNPs cause progressive deterioration of olfactory acuity, whereas a partial olfactory recovery was documented for continuous Cu^{2+} exposure. The present study strongly suggests the mechanism of toxicity caused by CuNPs is distinct from that of Cu^{2+} .

A1.16 INDUCTION CAPABILITY AND FUNCTIONALITY OF THE ARYL HYDROCARBON RECEPTOR 2 (Ahr2) IN HIGH-ANTARCTIC NOTOTHENIROID FISH

📅 MONDAY 3 JULY, 2017 ⌚ 16:55

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The Aryl hydrocarbon receptor (Ahr) pathway is a common entry point for the biological activity of dioxin-like compounds. Currently, no information exists on the presence and functionality of the Ahr in Antarctic notothenioid fishes.

Several proteins have been lost in Antarctic fish. We aimed therefore to investigate if Antarctic notothenioids have conserved a functional Ahr. Specifically, we identified and cloned the Ahr2 sequence and tested its sensitivity to AhR model-agonists in vitrousing a luciferase reporter gene assay in which COS7-cells were transfected to express the Ahr2 ligand binding domain of two notothenioid species. The red-blooded *Trematomus loennbergii* and the white-blooded *Chionodraco hamatus*, originating from the Weddell Sea, served as models.

We firstly demonstrated that Antarctic fish express Ahr2 in liver and found that the Ahr2 of both species was activated by AhR agonists (e.g. Benzo(a)pyrene, 6-formylindolo[3,2-b]carbazole (FICZ), beta-Naphtoflavone & dioxine-like PCBs) in the ligand binding assay. The activation was similar or only 50% in the Antarctic fish compared to the response of the Atlantic cod (*Gadus morhua*) ligand-binding domain, which served as reference.

Our study revealed that Ahr2 in (the investigated) Antarctic fish have retained the ability of being ligand-activated, and could potentially induce the Ahr2-mediated toxicant metabolism. We thus conclude that Antarctic fish might have the capability to handle anthropogenic pollutants, yet differences in the induction capability and thus xenobiotics metabolism rate may exist to temperate zone fish.

A1.17 PHYSIOLOGICAL EFFECTS AND BIOMARKERS OF DILUTED BITUMEN EXPOSURE IN EARLY LIFE STAGE SOCKEYE SALMON

MONDAY 3 JULY, 2017 17:10

SARAH L ALDERMAN (UNIVERSITY OF GUELPH, CANADA), FENG LIN (SIMON FRASER UNIVERSITY, CANADA), LAURA A DINDIA (UNIVERSITY OF GUELPH, CANADA), ANTHONY P FARRELL (UNIVERSITY OF BRITISH COLUMBIA, CANADA), CHRISTOPHER J KENNEDY (SIMON FRASER UNIVERSITY, CANADA), TODD E GILLIS (UNIVERSITY OF GUELPH, CANADA)

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Canada has the world's third largest crude oil reserve, most of which is bitumen - a heavy, tar-like crude from the Alberta oil sands. Extracted bitumen is diluted (dilbit) to permit flow through thousands of kilometers of pipelines that traverse North America, crossing numerous waterways including natal streams of Pacific salmon. Crude oil exposure is particularly toxic to early life stage fish and the developing heart; therefore accidental dilbit release into salmon habitat could have a major impact on these migratory fish. We exposed sockeye salmon to different concentrations of the water-soluble fraction of dilbit at two early life stages (embryos and parr), and then quantified physiological endpoints relevant to migration and the development of bio-monitoring tools. We found that swimming performance and cardiac histology were impacted in fish exposed to dilbit as parr; but swimming performance was not affected in fish exposed to dilbit as embryos and then raised in clean water to the parr stage. We quantified changes in the serum proteome following dilbit exposure and identified several candidate biomarkers that increased independent of exposure length (1 wk or 4 wk) or exercise treatment. In addition, increased abundances of intracellular proteins, including contractile proteins, were present in serum from exposed and exercised parr, suggesting that dilbit exposure exacerbates exercise-induced tissue damage and may compromise the ability of salmon to sustain and recover from intense exercise. This study provides insight into dilbit toxicity in a socioeconomically important fish. *Supported by the National Contaminants Advisory Group, Fisheries and Oceans Canada.*

A1.10 THE ULTIMATE TOXICOLOGICAL MIXTURE: EFFECTS OF HYDRAULIC FRACTURING FLUID ON MODEL FRESHWATER SPECIES

TUESDAY 4 JULY, 2017 POSTER SESSION

TAMZIN BLEWETT (UNIVERSITY OF ALBERTA, CANADA), PERRINE DELOMPRE (UNIVERSITY OF ALBERTA, CANADA), YUHE HE (UNIVERSITY OF ALBERTA, CANADA), ERIK FOLKERTS (UNIVERSITY OF ALBERTA, CANADA), SHANNON FLYNN (UNIVERSITY OF ALBERTA, CANADA), DANIEL ALESSI (UNIVERSITY OF ALBERTA, CANADA), GREG GOSS (UNIVERSITY OF ALBERTA, CANADA)

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Hydraulic fracturing is an industrial process allowing for the extraction of gas or oil. To fracture the rocks, a proprietary mix of chemicals is injected under high pressure, which later returns to the surface as flowback and produced water (FPW). FPW is a complex chemical mixture consisting of trace metals, organic compounds, and often, high levels of salts. FPW toxicity to the model freshwater crustacean, *Daphnia magna*, was characterized utilizing acute (48 h median lethal concentrations; LC_{50}) and chronic (21 d) exposures. Neonates exhibited an LC_{50} of 0.19% of full-strength FPW, making them more sensitive than adults, which displayed an LC_{50} value of 0.75%. A decrease in reproduction was observed, with a mean value of 18.5 neonates produced per replicate over a 21-d chronic exposure to 0.04% FPW, significantly decreased from the average of 64 neonates produced in controls. The time to first brood was delayed in the highest FPW (0.04%) treatment. Quantitative PCR highlighted significant changes in expression of genes encoding xenobiotic metabolism (cyp4) and moulting (cut). This study is the first to characterize chronic FPW toxicity and will be used to help understand the impacts, and develop environmental monitoring and risk assessment, of FPW spills.

A1.11 EMERGING INSIGHTS IN OIL TOXICITY: EVIDENCE OF NON-CANONICAL IMPAIRMENT IN FISH

TUESDAY 4 JULY, 2017 POSTER SESSION

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Oil is a pervasive environmental contaminant in marine systems, epitomized by the 2010 Deepwater Horizon oil spill that released over 700 million litres of crude oil into the northern Gulf of Mexico. Decades of research on oil toxicity has helped shape a scientific consensus that lethal and sub-lethal effects of oil exposure are the result of impaired cardiac development and function, which can lead to reduced cardiac output, aerobic scope, swim performance and ultimately reduced growth, survival and fitness. Our recent work related to the Deepwater Horizon oil spill, while also demonstrating cardiotoxicity, has begun to reveal novel non-canonical toxicity endpoints of ecological significance that are as, or more, sensitive than cardiotoxicity. These include observed impairment in the development of brain and vision systems, as evidenced by high-throughput transcriptomic data combined with morphological

analysis. Acute oil exposure in later life stage animals also resulted in altered behaviors across a variety of species and experimental designs. These include reduced sociability and thigmotaxis, as well as altered settlement and habitat usage behavior. Overall, these observations are consistent with the hypothesis that oil exposure reduces anti-predator behavior, likely through impaired brain and sensory function, which can have clear ecological consequences for affected individuals. These data will be presented in the context of a novel adverse outcomes pathway that can be used to complement existing frameworks specific to oil induced cardiac impairment.

A1.11 EMERGING INSIGHTS IN OIL TOXICITY: EVIDENCE OF NON-CANONICAL IMPAIRMENT IN FISH

TUESDAY 4 JULY, 2017 POSTER SESSION

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Oil is a pervasive environmental contaminant in marine systems, epitomized by the 2010 Deepwater Horizon oil spill that released over 700 million litres of crude oil into the northern Gulf of Mexico. Decades of research on oil toxicity has helped shape a scientific consensus that lethal and sub-lethal effects of oil exposure are the result of impaired cardiac development and function, which can lead to reduced cardiac output, aerobic scope, swim performance and ultimately reduced growth, survival and fitness. Our recent work related to the Deepwater Horizon oil spill, while also demonstrating cardiotoxicity, has begun to reveal novel non-canonical toxicity endpoints of ecological significance that are as, or more, sensitive than cardiotoxicity. These include observed impairment in the development of brain and vision systems, as evidenced by high-throughput transcriptomic data combined with morphological analysis. Acute oil exposure in later life stage animals also resulted in altered behaviors across a variety of species and experimental designs. These include reduced sociability and thigmotaxis, as well as altered settlement and habitat usage behavior. Overall, these observations are consistent with the hypothesis that oil exposure reduces anti-predator behavior, likely through impaired brain and sensory function, which can have clear ecological consequences for affected individuals. These data will be presented in the context of a novel adverse outcomes pathway that can be used to complement existing frameworks specific to oil induced cardiac impairment.

A1.12 TOXICITY OF OIL SANDS PROCESS-AFFECTED WATER ON FEEDING, RESPIRATORY AND CIRCULATORY SYSTEMS OF *DAPHNIA MAGNA* AND ORGANISM LEVEL MANIFESTATIONS

TUESDAY 4 JULY, 2017 POSTER SESSION

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Oil sands process-affected water (OSPW), a byproduct of the extraction of bitumen in the surface-mining oil sands industry, is currently stored in massive on-site tailings ponds. Determining the potential effects of OSPW on aquatic ecosystems is of main concern to oil sands companies and legislators concerned about the reclamation of mining sites. In the first phase of the present study, the acute (i.e. 24h) and chronic (i.e. 10 days) effects of OSPW on some fundamental survival endpoints (feeding, oxygen consumption, heart rate, and hemoglobin content) of *Daphnia magna* was studied. The results of these experiments demonstrated that OSPW impairs the feeding, reduces hemoglobin content and increases oxygen consumption in *D. magna*. However, OSPW did not alter the heart rate in exposed animals. These results suggest that exposure to OSPW reduces energy absorption and increases energy expenditure in *D. magna*. Based on these results, we hypothesized that OSPW might reduce the fitness of *D. magna*. To investigate the effects of OSPW on fitness and population health, we examined growth, reproduction and macronutrient reserves of *D. magna* after chronic exposure to OSPW. The results of these experiments revealed that all three measured endpoints of the OSPW exposed animals were impaired. The outcomes of the current study demonstrated that OSPW affects individuals and population of *D. magna* by altering the energy balance (i.e. intake and demand).

A1.13 UPTAKE KINETICS AND SUBCELLULAR COMPARTMENTALISATION EXPLAIN LETHAL BUT NOT SUBLETHAL EFFECTS OF CADMIUM IN TWO CLOSELY RELATED AMPHIPOD SPECIES

TUESDAY 4 JULY, 2017 POSTER SESSION

LENA JAKOB (ALFRED WEGENER INSTITUTE HELMHOLTZ CENTRE FOR POLAR AND MARINE RESEARCH, GERMANY), DARIA BEDULINA (IRKUTSK STATE UNIVERSITY, RUSSIA), MICHAEL GINZBURG (ALFRED WEGENER INSTITUTE HELMHOLTZ CENTRE FOR POLAR AND MARINE RESEARCH, GERMANY), ZHANNA SHATILINA (IRKUTSK STATE UNIVERSITY, RUSSIA), YULIA LUBYAGA (IRKUTSK STATE UNIVERSITY, RUSSIA), EKATERINA MADYAROVA (IRKUTSK STATE UNIVERSITY, RUSSIA), ANTON GURKOV (IRKUTSK STATE UNIVERSITY, RUSSIA), MAXIM TIMOFEEV (IRKUTSK STATE UNIVERSITY, RUSSIA), HANS-OTTO PÖRTNER (ALFRED WEGENER INSTITUTE HELMHOLTZ CENTRE FOR POLAR AND MARINE RESEARCH, GERMANY), FRANZ JOSEF SARTORIS (ALFRED WEGENER INSTITUTE HELMHOLTZ CENTRE FOR POLAR AND MARINE RESEARCH, GERMANY), ROLF ALTENBURGER (UFZ - HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH, GERMANY), TILL LUCKENBACH (UFZ - HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH, GERMANY)

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This study aimed to compare the sensitivity to waterborne cadmium (Cd) of two closely related amphipod species endemic to Lake Baikal. Both display different body sizes and concentrations of cellular stress response (CSR) proteins. Higher Cd uptake rates by *Eulimnogammarus cyaneus* than by *Eulimnogammarus verrucosus* (individual weight: 20-44 and 418-942 mg, respectively) in concentration-mortality-studies (4 week; 6°C) explained that lethal concentrations were lower in *E. cyaneus* than in *E. verrucosus*. When exposed to a Cd concentration inducing 1% of mortality (LC1; *E. cyaneus*: 18 nM and *E. verrucosus*: 115 nM), the metal sensitive tissue fractions of Cd (MSF) were similar in *E. verrucosus* and *E. cyaneus* (0.26±0.07 and 0.25±0.06 µg/g fresh weight⁻¹, respectively). In accordance with the higher constitutive concentration of CSR proteins in *E. cyaneus* than in *E. verrucosus*, more Cd was biologically detoxified in the former species (*E. cyaneus* = 0.83±0.13 and *E. verrucosus* = 0.66±0.1 µg/g (fresh weight)⁻¹). LC1 exposure induced decreased respiration and ventilation rates (by 15 to 38%) at all sampling time points only in *E. verrucosus* but not in *E. cyaneus*. The physiological trait of depressed metabolism, which has evolved to endure adverse conditions in the short-term, might turn detrimental during persistent exposure to toxicants.

A1.18 EVOLUTION AND EXPRESSION OF THE METAL RESPONSE IN THE ASIAN LANCELET

TUESDAY 4 JULY, 2017 POSTER SESSION

THOMAS SORGER (ROGER WILLIAMS UNIVERSITY, UNITED STATES), LEA KABLIK (ROGER WILLIAMS UNIVERSITY, UNITED STATES), CHRIS MATERNA (ROGER WILLIAMS UNIVERSITY, UNITED STATES), ALICE CHAN (CITY UNIVERSITY OF HONG KONG, HONG KONG), PAUL SHIN (CITY UNIVERSITY OF HONG KONG, UNITED STATES)

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Lancelets are basal chordates found in shallow, coastal habitats that are highly susceptible to ocean warming and pollution. In vertebrates and invertebrates exposed to trace metals, metal-activated transcription factor 1 (MTF1) induces the expression of *metallothionein* (Mth). We investigated (a) the evolution of these proteins in three *Branchiostoma* species and (b) their role in the response of the Asian lancelet, *B. belcheri*, to the trace metals Cd, Ni and Cr.

(a) The MTF1 genes in *B. belcheri*, *B. lanceolatum* and *B. floridae* encode 6 C₂H₂-type zinc fingers, same as the domain architecture in vertebrates. Bayesian phylogeny confirms the basal position of cephalochordates and indicates that MTF1 in the Asian lancelet diverged earliest from the ancestral chordate gene. (b) Exposure for 72h to 100 ppb Cd or Ni led to significant increases in Mth/actin mRNA (35-fold and 24-fold, respectively) and MTF1/actin mRNA (75-fold and 22-fold, respectively). The order of activity of these metals, Cd > Ni > Cr = Control, corresponds to the predicted ability of their hydrated ions to compete for a zinc binding site (p < 0.01).

Thus the Asian lancelet genome encodes a conserved MTF1, and low concentrations of Cd or Ni cause a significant induction of Mth mRNA. Regarding the surprising increase in MTF1 expression, the promoter regions in both the Asian lancelet and zebrafish appear to lack a conserved metal response element (MRE), so upregulation by metals of this transcription factor may reflect an increase in mRNA stability, rather than an increase in *de novo* transcription.

A1.19 PRIMARILY CULTURED GILL EPITHELIA AS PROTOTYPES FOR ASSESSING FISH RESPONSE TO HEAVY METAL EXPOSURE

TUESDAY 4 JULY, 2017 POSTER SESSION

☎ KAFILAT A BAWA-ALLAH (UNIVERSITY OF LAGOS, NIGERIA), ADEBAYO A OTITOLUJU (UNIVERSITY OF LAGOS, NIGERIA), JOSEPH K SALIU (UNIVERSITY OF LAGOS, NIGERIA), NIC R BURY (KINGS COLLEGE LONDON, UNITED KINGDOM), LUCY C STOTT (KINGS COLLEGE LONDON, NIGERIA)

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This study investigated the use of primarily cultured gill cells to assess changes in gill physiology in response to heavy metal exposure. Rainbow trout (*Oncorhynchus mykiss*) gill epithelia were cultured on permeable filter supports using a Double Seeded Insert (DSI) primary culture technique. The cells, which are tolerant to freshwater application on the apical surface, were exposed to a range of concentrations of zinc (Zn) [1-100 µM], lead (Pb) [0.5-50 µM] and cadmium (Cd) [0.01-1.0 µM] for 24h. The expression of heavy metal responsive genes metallothionein A (mtA) and B (mtB) were quantified using Reverse Transcription quantitative Polymerase Chain Reaction (RT qPCR). Results showed that Zn significantly ($P < 0.05$) enhanced the expression of mtA and mtB in the cultured gill epithelia while Pb significantly ($P < 0.05$) inhibited the expression of mtA. These findings corroborate previous *in vivo* studies which showed that increased production of metallothionein in fish is associated with an increase in internal concentrations of Cadmium, Mercury (Hg), Copper (Cu) and Zn only. The global call to reduce the number of fish used in toxicological evaluations has necessitated the need to develop *in vitro* systems as viable alternatives. This study demonstrated that primarily cultured gill epithelia is capable of detecting bioavailable metals in water and thus shows promise as a surrogate for fish toxicity tests.

A1.20 THE PHYSIOLOGICAL EFFECTS OF POLYETHYLENE MICROBEADS INGESTION IN JUVENILE ORANGE-SPOTTED GROUPER (*EPINEPHELUS COIOIDES*)

TUESDAY 4 JULY, 2017 POSTER SESSION

☎ PEI-CHI CHUNG (NATIONAL TAIWAN OCEAN UNIVERSITY, TAIWAN), YUNG-CHE TSENG (INSTITUTE OF CELLULAR AND ORGANISMIC BIOLOGY ACADEMIA SINICA, TAIWAN), YI TA SHAO (NATIONAL TAIWAN OCEAN UNIVERSITY, TAIWAN)

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Plastic particle in the ocean is an emerging pollution that affect aquatic habitats globally. Some of them are very small that can be eaten by a large range of animals, including fishes. However, the physiological influence on fish that ingested those microscopic particles is still unclear. This study aimed to investigate the physical impact of polyethylene (PE 0.2-125µm) microbeads ingestion in juvenile orange-spotted grouper (*Epinephelus coioides*). To this end, treatment fish were fed with the diet including 10^4 fluorescent microbeads per gram for two weeks. The remaining beads were

searched and counted in the gastrointestinal tract and liver using fluorescent microscope. Furthermore, the possible stress and immune responses were estimated by the changing in transcripts of head kidney *cyp11a1* (P450_{scc}) and *star* (steroidogenic acute regulator), and hepatic *lept* (leptin) genes. As well as, the plasma ROS and cortisol levels will be determined for the possible immune responses.

A1.22 THE EFFECT OF THERMAL PREHISTORY AND EXPOSURE REGIME ON METAL TOXICITY TOLERANCE IN ZEBRAFISH (*DANIO RERIO*)

TUESDAY 4 JULY, 2017 POSTER SESSION

☎ ALI PILEHVAR (UNIVERSITY OF ANTWERP, BELGIUM), RONNY BLUST (UNIVERSITY OF ANTWERP, BELGIUM), KATHERINE CORDERY (UNIVERSITY OF ANTWERP, BELGIUM)

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Temperature is one of the most important environmental factors driving physiological and ecological dynamics. Although the effect of temperature on energy metabolism and other physiological processes has been subject of detailed study the effect of temperature on the sensitivity of environmental toxicants is poorly documented. Moreover, the effect of temperature is generally not considered in the setting of environmental quality standards. In this work we explore the effect of temperature acclimation and exposure on the uptake and toxicity of Cu and Cd and their mixtures in the zebrafish. To this end, we have defined 3 main scenarios including a short temperature treatment (1-4 days) in 17, 22, 25, 32 and 34°C and subsequent metal exposure for 10 days at the optimal temperature (Scenario 1), the same but with a 28 days temperature acclimation (Scenario 2) and a 28 days temperature acclimation followed by metal exposure at the temperature of acclimation (Scenario 3). The results showed that Cu was much more toxic than Cd. However, Cu and Cd together showed a strong synergistic effect. A short low temperature treatment (Scenario 1) appears to be protective while a short high temperature treatment increases sensitivity. In contrast, in Scenarios 2 and 3 higher temperatures increase the tolerance against metal toxicity. Although the temperature regime had a significant effect on metal accumulation there was no clear relationship between metal accumulation and toxicity. The results clearly show the importance of temperature in modulating the toxicological effects of metals and their mixtures in the zebrafish.

A1.23 ZINC TROPHIC TRANSFER IN FISH: AN INTEGRATIVE ASSESSMENT OF THE ROLE OF FOOD QUALITY, FEEDING FREQUENCY AND ENVIRONMENTAL CONDITIONS

TUESDAY 4 JULY, 2017 POSTER SESSION

● SIMON POUIL (UNIVERSITY OF LA ROCHELLE, FRANCE), PACO BUSTAMANTE (UNIVERSITY OF LA ROCHELLE, FRANCE), FRANÇOIS OBERHÄNSLI (INTERNATIONAL ATOMIC ENERGY AGENCY, MONACO), PETER SWARZENSKI (INTERNATIONAL ATOMIC ENERGY AGENCY, MONACO), MARC METIAN (INTERNATIONAL ATOMIC ENERGY AGENCY, MONACO)

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A mechanistic approach provides a realistic assessment of how environmental factors impact the digestive physiology of fish, which may have implications for trophic transfer studies. However, only an integrative approach can experimentally confirm whether food compound assimilation is actually impacted by single or multiple changes in environmental conditions (i.e., both abiotic and biotic factors). Zinc (Zn) is mainly acquired by fish through food ingestion, and its transfer has been intensively investigated in ecotoxicological studies as it is both an essential dietary element but can also be toxic at higher levels. In order to understand the effects of abiotic and biotic factors on the Zn assimilation efficiency (AE) by fish, a series of experimental studies have been carried out using radiotracer techniques. After feeding with a ⁶⁵Zn-radiolabeled food source, Zn depuration kinetics were followed in different fish species and under varying experimental factors, including food type and pH and temperature changes. Results obtained from five sets of experiments suggest that Zn assimilation in fish is subject to inter-specific differences. In addition, Zn AE for a given species, is mainly influenced by the type of food rather than by abiotic factors. This limited impact of seawater parameters on Zn trophic transfer is particularly important in the context of Zn physiology or ecotoxicology in fish subjected to expected global change scenarios, that will include a combination of stressors.

A1.24 PERSISTENT ORGANIC POLLUTANTS IN LOW- VS. HIGH-ANTARCTIC NOTOTHENIIDS

TUESDAY 4 JULY, 2017 POSTER SESSION

● ANNELI STROBEL (MAN-SOCIETY-ENVIRONMENT UNIVERSITY OF BASEL, SWITZERLAND), PETER SCHMID (EMPA LABORATORY FOR ADVANCED ANALYTICAL TECHNOLOGIES, SWITZERLAND), PATRICIA BURKHARDT-HOLM (MAN-SOCIETY-ENVIRONMENT UNIVERSITY OF BASEL, SWITZERLAND), HELMUT SEGNER (CENTRE FOR FISH AND WILDLIFE HEALTH UNIVERSITY OF BERNE, SWITZERLAND), MARKUS ZENNEGG (EMPA LABORATORY FOR ADVANCED ANALYTICAL TECHNOLOGIES, SWITZERLAND)

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It has been suggested that High-Antarctic waters, remote from human activities, are impacted by anthropogenic pollution, and that the local biota are accumulating the contaminants. At present, no data exist on persistent organic pollutant (POP) body burdens for nototheniids inhabiting the High-Antarctic Weddell Sea. We determined the pollutant load in muscle tissue of red- and white-blooded (*Trematomus loennbergii* and *Chionodraco hamatus*, respectively) fish from the Weddell Sea. We compared these data to our previous measurements of POPs in Low-Antarctic nototheniids. We analysed concentrations of various organochlorine pesticides (OCPs), polychlorinated biphenyls (indicator (i) PCBs, dioxine-like (dl) PCBs and polybrominated diphenyl ethers (PBDEs), and calculated 2,3,7,8-TCDD toxic equivalents (TEQs).

We detected lower levels of β -HCH and Σ iPCBs in *C. hamatus* compared to *T. loennbergii*, and higher levels Σ PBDEs (per lipid weight) in *T. loennbergii* than in *C. hamatus*. Body burdens were mostly similar to those of Low-Antarctic fish, and not related to the trophic positions of the species. The TEQs of the High-Antarctic species were similar in *C. hamatus*, *T. loennbergii* and the Low-Antarctic nototheniids.

The variations we observed in POP levels between and within High- and Low-Antarctic nototheniids did not correspond to the sampling site, ecological differences or trophic levels of the species, but might rather be related to species and metabolic effects. The present findings suggest that fishes of High-Antarctic waters, although this area is more remote and less influenced by local human activities, do not show clearly lower POP body burdens than fishes from Low-Antarctic waters.

A1.25 IMPACTS OF CRUDE OIL ON CARDIOMYOCYTE FUNCTION IN THE MAHI-MAHI (*CORYPHAENA HIPPURUS*)

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 RACHAEL M HEUER (UNIVERSITY OF MIAMI-RSMAS, UNITED STATES), HOLLY A SHIELS (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), GINA LJ GALLI (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), GEORGINA K COX (UNIVERSITY OF MIAMI-RSMAS, UNITED STATES), JOHN D STIEGLITZ (UNIVERSITY OF MIAMI-RSMAS, UNITED STATES), DANIEL D BENETTI (UNIVERSITY OF MIAMI-RSMAS, UNITED STATES), MARTIN GROSELL (UNIVERSITY OF MIAMI-RSMAS, UNITED STATES), DANE A CROSSLEY II (UNIVERSITY OF NORTH TEXAS, UNITED STATES)

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The Deepwater Horizon oil spill occurred in areas of the Gulf of Mexico that overlap with the habitat of the mahi. Crude oil has been demonstrated to have adverse impacts on cardiovascular function across multiple levels of biological organization. At the organ level, *in situ* preparations have revealed that crude oil causes a reduction in cardiac output that likely accounts for reductions in whole-animal performance, including reduced maximal sustained swimming speed and reduced maximal metabolic rate. On this background, we studied the underlying causes of impaired cardiovascular function by examining the impacts of oil on mahi cardiomyocytes. At the cellular level of organization, excitation-contraction coupling consists of three main events: (1) the generation of an action potential, (2) an increase of intracellular calcium, which causes (3) cellular contraction. The first two components of this cycle have been studied in pelagic fish but direct measurement of oil impacts on contractility have yet to be reported in oil-exposed teleost myocytes. In the present study, ventricular cardiomyocytes were perfused with control extracellular saline or extracellular saline containing one of three levels of oil (3.2, 7.2, 15.4 μ PAH). Sarcomere shortening and other aspects of the contractility were measured using an IonOptix real-time cellular recording system. Cells exposed to oil experienced a reduction in sarcomere shortening, an impact that appears to be exacerbated at higher stimulation frequencies, supporting observations of reductions in whole-animal performance. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520.

A1.26 DIFFERENTIAL EFFECTS OF SUB-LETHAL COPPER AND NICKEL CONCENTRATIONS ON OLFACTORY SENSITIVITY IN THE MOZAMBIQUE TILAPIA (*OREOCHROMIS MOSSAMBICUS*)

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 PETER C HUBBARD (CENTRO DE CIÊNCIAS DO MAR, PORTUGAL), ADELINO VM CANÁRIO (CENTRO DE CIÊNCIAS DO MAR, PORTUGAL)

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The olfactory system of fish is vital for many biological processes, including chemical communication, predator avoidance and food-search. The intimate contact of olfactory sensory neurones with the medium means that they are particularly vulnerable to pollutants - such as heavy metals - in the water, even at sub-lethal concentrations. However, the mechanisms of toxicity are not always clear. The current study used the near-unique sigmoidal concentration-response curve (as assessed by the electro-olfactogram) of the tilapia to its steroidal pheromone 5 α -pregnane-3 α ,17 α ,20 β -triol-3 α -glucuronate to differentiate between non-specific cytotoxicity and direct effects on ligand-receptor affinity of copper and nickel, two common anthropogenic toxicants in freshwater habitats. Low copper concentrations (3-100 nM) caused progressive reduction in the maximum olfactory response (I_{max}), but apparent affinity (EC_{50}) and Hill co-efficient were unaffected (except at 100 nM copper). In contrast, low nickel concentrations (1-30 μ M) caused a progressive reduction in receptor-ligand affinity (increased apparent EC_{50} , decreased Hill co-efficient) while the I_{max} was unaffected. Thus, copper reduces olfactory sensitivity via non-specific cytotoxicity and/or interference with olfactory transduction pathway(s), whereas nickel causes a reduction of affinity of the olfactory receptor for its ligand, possibly by binding to the extracellular region of the receptor and causing a conformational change in the ligand-binding domain. Interestingly, relatively high concentrations of NaCl (100 mM; the tilapia is euryhaline) caused a similar reduction in apparent receptor-ligand affinity, possibly by altering the charge distribution within the ligand-binding domain. We believe that this experimental model will prove valuable for examining G-protein coupled receptor function *in vivo*.

A1.27 STREAM-LINING THE ADOPTION OF ENVIRONMENTAL REGULATIONS ACROSS BIOMES: THE IMPORTANCE OF FUNDAMENTAL PHYSIOLOGICAL KNOWLEDGE

TUESDAY 4 JULY, 2017 POSTER SESSION

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Fish are a large and diverse group, displaying a vast range of physiological traits. Although their responses to toxicants are shaped by their physiology, our mechanistic understanding of this relationship (a key component of current environmental protection regulations), has largely been restricted to a few, Northern Hemisphere species. Using the widespread Southern Hemisphere fish inanga (*Galaxias maculatus*), the toxic mechanisms of a range of environmental contaminants, including metals (e.g. copper, zinc, nickel) and pharmaceuticals (e.g. diclofenac), have been characterised. In general, mechanisms of toxicity in inanga are conserved relative to better-studied species, confirming the general principles underlying predictive approaches to environmental protection. However, inanga display some significant differences in toxic responses, which may be attributed to their unusual physiology. This research highlights the importance of understanding the fundamental biology of indigenous species when applying environmental regulations across biomes.

A1.28 THE INFLUENCE OF OIL EXPOSURE ON SOCIAL INTERACTIONS AND COMPETITION IN A MARINE TELEOST

TUESDAY 4 JULY, 2017 POSTER SESSION

ALEXIS J KHURSIGARA (UNIVERSITY OF TEXAS AT AUSTIN, UNITED STATES), JACOB L JOHANSEN (UNIVERSITY OF TEXAS AT AUSTIN, UNITED STATES), ANDREW J ESBAUGH (UNIVERSITY OF TEXAS AT AUSTIN, UNITED STATES)

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In resource-limited environments, fish establish social hierarchies in which some fish become dominant while others become subordinate. Dominant fish experience reduced social stress while monopolising resources, and have higher fitness and survival. Subordinate fish experience chronic social stress that leads to a cascade of physiological impairments that can contribute to ecological death. Following the Deepwater Horizon oil spill, a number of studies on species native to the Gulf of Mexico demonstrated that physiological indices such as aerobic scope and swim performance are reduced following exposure to low concentrations of polycyclic aromatic hydrocarbons (PAHs). Building on these findings, we have previously shown that oil exposure results in social subordination in a dyad setting. Based on this evidence, we hypothesised that oil exposed individuals would be at a competitive disadvantage in larger group settings, which would result in reduced growth and elevated chronic stress indicators. While no differences were observed in an 8 week growth test utilising satiating feeding regimes, when

resources were limited, individuals exposed to environmentally realistic concentrations of oil showed reduced specific growth rate. Furthermore, the highest exposure concentration showed a significantly reduced standard metabolic rate. These data demonstrate that the physiological impairments imposed by sub-lethal oil exposure can impact downstream ecological performance.

A1.30 INSIGHTS INTO THE GENETIC BASIS OF DRUG RESISTANCE OF THE SALMON LOUSE (*LEPEOPHTHEIRUS SALMONIS*)

TUESDAY 4 JULY, 2017 POSTER SESSION

ARMIN STURM (UNIVERSITY OF STIRLING, UNITED KINGDOM), GRETA CARMONA-ANTOÑANZAS (UNIVERSITY OF STIRLING, UNITED KINGDOM), DAVID GUIDI (UNIVERSITY OF STIRLING, UNITED KINGDOM), ROSS D HOUSTON (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), KARIM GHARBI (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), MICHAËL BEKAERT (UNIVERSITY OF STIRLING, UNITED KINGDOM), JAMES E BRON (UNIVERSITY OF STIRLING, UNITED KINGDOM)

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Globally, insecticides are used widely for the control of arthropod disease vectors, parasites and phytophagous pests. However, long-term use of chemical control agents can result in the evolution of resistance. Caligid sea lice are copepod ectoparasites of marine fish causing substantial economic losses in the commercial mariculture of Atlantic salmon. While non-chemical methods are increasingly implemented for sea louse control, the salmon farming industry still relies widely on treatments with veterinary delousing agents. However, resistance has been reported for different anti-sea louse drugs including the macrocyclic lactone emamectin benzoate (EMB) and the pyrethroid deltamethrin (DM). The aim of this study was to analyse the genetic basis of EMB and DM resistance of the caligid *Lepeophtheirus salmonis* (salmon louse). Reciprocal crosses were performed between two *L. salmonis* strains differing 10-fold in EMB and 140-fold in DM susceptibility. F1 siblings were crossed to produce F2 parasites, which were characterised regarding their drug susceptibility before being sampled for DNA extraction. F2 salmon lice showed a wider range of EMB susceptibilities ranging from fully susceptible to fully resistant. Double digest restriction site-associated DNA (ddRAD) sequencing was used for the discovery and genotyping of genome-wide single nucleotide polymorphisms (SNP). Three QTL regions involved in EMB resistance were identified. In contrast, in families derived from a resistant P0 dam all F2 animals were DM resistant while in the reciprocal families only few F2 animals were DM resistant. The results provide evidence of roles of maternally transmitted factors in the pyrethroid resistance of *L. salmonis*.

A1.31 RED AND BLUE, WHAT WILL YOU DO?

■ TUESDAY 4 JULY, 2017 POSTER SESSION

👤 ADISON K ADAMS (UNIVERSITY OF NORTH TEXAS, UNITED STATES), WREN A BUSBY (UNIVERSITY OF NORTH TEXAS, UNITED STATES), IONE HUNT VON HERBING (UNIVERSITY OF NORTH TEXAS, UNITED STATES), BENNY GONZALEZ (UNIVERSITY OF NORTH TEXAS, UNITED STATES)

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Larval Red Drum (*Sciaenops ocellatus*) pass through a series of developmental stages post-hatching. The "larval stage" is defined from the point of hatching to the complete attainment of fin ray counts (Kendall, 1984). One of the issues with growing larval fish in a tank setting, instead of "in the wild" is the possibility of infections on the eggs. Many industries rinse the eggs in antibiotic solution prior to placement in the tanks (Westerfield, M. 2007). This is a problem because fish build up intestinal microfauna from their environment, and studies have shown how important a more diverse gut colony is in development (Hansen and Olafsen, 1999). With the use of antibiotics, we are clearing the naturally occurring bacteria from the environment, which leads to a delay in the development of the enteric gut and "second brain" (Goldstein et al., 2012). The delayed development of the gut, can lead to nutritional deficiencies, which can lead to developmental delays in bone and bone muscle of fish (Cahu, et al., 2003). Through two methods of staining, using alizarin red and alcian blue, we are showing the changes in development in both cartilage and bone after the treatment of either antibiotics or probiotics till 28 days post hatch. Also, determining if the two stains can be used sequentially, or if a negative interaction between the two stains disrupts the staining of either cartilage or bone. This study will help produce a more inclusive staining protocol for future experiments and histological examinations.

A1.32 THE CYTOCHROME P450 SUPERFAMILY OF THE SALMON LOUSE (*LEPEOPHTHEIRUS SALMONIS*)

■ WEDNESDAY 5 JULY, 2017 POSTER SESSION

👤 ARMIN STURM (UNIVERSITY OF STIRLING, UNITED KINGDOM), GRETA CARMONA-ANTOÑANZAS (UNIVERSITY OF STIRLING, UNITED KINGDOM), JOSEPH L HUMBLE (UNIVERSITY OF STIRLING, UNITED KINGDOM), MICHAËL BEKAERT (UNIVERSITY OF STIRLING, UNITED KINGDOM), JAMES E BRON (UNIVERSITY OF STIRLING, UNITED KINGDOM)

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The superfamily of cytochrome P450 monooxygenases (CYPs) consists of heme-thiolate proteins, which in arthropods are involved in the metabolism of juvenile hormones and ecdysteroids, as well as exogenous compounds. The overexpression of CYPs is commonly observed in insecticide resistance. The salmon louse (*Lepeophtheirus salmonis*) is an ectoparasite infecting marine salmonids and causing great economic losses in industrial salmon farming. Despite the increasing implementation of non-chemical salmon delousing, veterinary drugs are still used widely to control the parasite. However, resistance formation has been reported, at least locally, for most control agents. The aim of this study was to provide a

survey of the genome-wide complement of CYPs in *L. salmonis* and assess potential roles of CYPs in the resistance against veterinary parasiticides. Homology searches of the *L. salmonis* genome resulted in the identification of 27 CYPs which were assigned to three clans according to the current nomenclature. Compared to the free-living copepod *Paracyclopina nana*, which possesses 46 CYPs, *L. salmonis* shows a markedly reduced number of CYP genes, which could correlate to its parasitic lifestyle resulting in a reduced exposure to environmental toxicants. RT-qPCR was used to study transcript abundance of CYPs in adult male and preadult-II female *L. salmonis* of a drug-susceptible strain and a multi-resistant strain characterised by decreased susceptibility to pyrethroids, organophosphates and avermectins. Transcript abundance of different CYPs showed sex differences or was enhanced following sublethal exposure to anti-sea louse agents (deltamethrin, emamectin benzoate) or ecdysteroids. In contrast, strain differences in CYP expression were lacking or comparatively minor.

A1.33 ECOTOXICOLOGICAL ASSESSMENT OF NOVEL POTENTIAL FORMICIDE: COMPARING *IN VITRO* AND *IN VIVO* CYTOTOXIC AND GENOTOXIC EFFECTS IN FISH HEPATOCYTES

■ TUESDAY 4 JULY, 2017 POSTER SESSION

👤 MARINA M BONOMO (FEDERAL UNIVERSITY OF SÃO CARLOS, BRAZIL), FELIPE ROCCO BLASCO (DEPARTMENT OF PHYSIOLOGICAL SCIENCES, FEDERAL UNIVERSITY OF SÃO CARLOS, SÃO PAULO, BRAZIL), JOÃO B FERNANDES (FEDERAL UNIVERSITY OF SÃO CARLOS, BRAZIL), MARISA N FERNANDES (FEDERAL UNIVERSITY OF SÃO CARLOS, BRAZIL)

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Continuous investments for pest control brings high relevance to development of environmental compounds to reduce use of pesticides with high ecological impact. However, applied chemicals can be indirectly released into larger, ecologically important water bodies, influencing surrounding biota. This study evaluates cytotoxic and genotoxic effects of a novel potential pesticide (MgPhen(Hesp)₂) through *in vitro* (*Danio rerio* hepatocyte cell line ZF-L) and *in vivo* (*Prochilodus lineatus* hepatocytes) methodologies. A clear impact on ZF-L cell population was noted 24 h after MgPhen(Hesp)₂ exposure when evaluating confluence/morphology, cell density, mitochondrial and lysosomal effects. Otherwise, increases in apoptosis/necrosis mechanisms, LDH enzyme leakage, membrane integrity and DNA break damages were not observed. *In vivo* analysis was also inconclusive, showing that breaks in genetic material were not a consequence of MgPhen(Hesp)₂ exposure. On the other hand, organ integrity was visually compromised with increases in concentrations. Therefore, observed effects could be either an influence in several cellular compartments leading to viability compromising or activation of non-apoptotic mechanisms, involving processes such as loss of mitochondrial function, ultimately leading to bioenergetics crisis and cell death. Elucidating pathways affected through different assays is a crucial step, since different exposure conditions may not exhibit same effects in a given target but can contribute to a final cellular response. Particularly when handling with novel chemicals, assessment of cytological parameters can be used as start to understand mechanisms of cellular responses. Thus, further studies on liver cells are extremely important to point out integration between *in vitro* and *in vivo* responses.

A1.34 MITIGATION OF CHEMICAL FLOCCULATION TOXICITY WITH A PROPRIETARY MITIGATION AGENT

TUESDAY 4 JULY, 2017 POSTER SESSION

ALEXANDER M CLIFFORD (UNIVERSITY OF ALBERTA, CANADA), EDYTA J JASINSKA (UNIVERSITY OF ALBERTA, CANADA), G HANNA (UNIVERSITY OF ALBERTA, CANADA), GREG G GOSS (UNIVERSITY OF ALBERTA, CANADA)

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Industrial operations such as mining, road building and aggregate washing result in high concentrations of suspended particles (Total Suspended Solids; TSS) in effluent waters. Tailing/settling ponds promote sedimentation of TSS and prevent adverse effects of sediment deposition in aquatic environments. Use of chemical coagulants/flocculants improves the efficacy of TSS sedimentation; however, these flocculants themselves have high toxic potential at very low concentrations (0.3-0.5 mg/L) including gill adherence and subsequent damage. A relatively novel practice is to treat flocculated water with a proprietary mitigation agent (MA), eliminating the toxicity of discharged water. However, the mechanism has not been empirically demonstrated. Given that exposure to the flocculent causes gill agglomeration and damage, we hypothesized that the mode of toxicity was via impairment of oxygen transport. We exposed fingerling trout (*Oncorhynchus mykiss*) to either flocculant (0.5 mg/L), MA (0.75 mg/L), or a mixture of the two and measured O₂ consumption over a 48h period. Furthermore, we excised gill for light microscopy (LM) and plasma, brain and liver tissue for analysis of tissue fuel (glucose/glycogen, ATP, phosphocreatine) and metabolites (lactate) to confirm the mode of flocculent toxicity and verify that mitigation causes no adverse sub-lethal effects. LM confirmed flocculent gill adherence and damage in the flocculent exposure group. When exposed to flocculant alone, trout presented ~50% reduction in O₂ consumption compared to both pre-exposure conditions and pairwise MA alone and in combination with flocculent. Our results demonstrate the mode of toxicity of flocculent exposure is via hypoxemia and demonstrate the efficacy of the MA.

A1.35 ALL OILED UP: THE EFFECTS OF CRUDE OIL ON CARDIOVASCULAR FUNCTION

WEDNESDAY 5 JULY, 2017 POSTER SESSION

GEORGINA K COX (UNIVERSITY OF MIAMI, UNITED STATES), DANE A CROSSLEY II (UNIVERSITY OF NORTH TEXAS, UNITED STATES), RACHAEL M HEUER (UNIVERSITY OF MIAMI, UNITED STATES), JOHN D STIEGLITZ (UNIVERSITY OF MIAMI, UNITED STATES), DANIEL D BENETTI (UNIVERSITY OF MIAMI, UNITED STATES), MARTIN GROSELL (UNIVERSITY OF MIAMI, UNITED STATES)

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Aqueous crude oil spills expose many fish species to varying concentrations of dissolved polycyclic aromatic hydrocarbons (PAH's), toxic components of oil, which have numerous lethal and sub-lethal effects. In particular, the heart is vulnerable to PAH toxicity which causes developmental cardiac abnormalities and impaired myocyte function. However, the cardiac responses of adult fish to acute oil exposure remain poorly understood. The aim of the current study was to assess cardiac function in a pelagic fish species, the coho (Rachycentron canadum), following acute (24 hr) exposure to ecologically relevant levels of dissolved PAH's. Cardiac power output (CPO), the product of ventricle pressure generation and volume output was used to quantify cardiovascular performance using an in situ heart preparation. Cardiovascular performance was varied using the β -adrenoceptor agonist isoproterenol (ISO) and by controlling afterload pressures. As expected, oil exposure adversely affected CPO with control fish achieving maximum CPO's (4 mW g⁻¹ Mv) greater than that of oil exposed fish (1 mW g⁻¹ Mv) at ISO concentrations of 1 x 10⁻⁶ M. However, saturation of β -adrenoceptors with ISO (1 x 10⁻⁵ M) rescued cardiac function. This indicates that in a natural setting increased plasma catecholamines could play a compensatory role that may mitigate some adverse effects of oil exposure in vivo.

This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).

A2 EFFECTS OF PHARMACEUTICALS ON WILDLIFE - BRIDGING THE GAP BETWEEN ECOTOXICOLOGY AND ECOLOGY

ORGANISED BY: JOSEFIN SUNDIN (UPPSALA UNIVERSITY, SWEDEN)
AND MIRJAM AMCOFF (STOCKHOLM UNIVERSITY, SWEDEN)

SESSION SPONSORED BY: THE COMPANY OF BIOLOGISTS

A2.1 PHARMACEUTICALS IN WILDLIFE - WHAT WE KNOW, WHAT WE DON'T KNOW, AND SHOULD WE WORRY?

📅 TUESDAY 4 JULY, 2017 ⌚ 10:30

👤 JUDIT E G SMITS (FACULTY OF VETERINARY MEDICINE
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Considering pharmaceuticals in the environment, concerns of ecologists and ecotoxicologists exist on multiple levels, the most obvious being biological effects on aquatic organisms such as fish, shellfish, and the organisms on which they feed. Human medications almost invariably end up in the aquatic system through urinary and fecal excretion of prescription and recreational drugs, as well as from improper disposal of unused drugs. There are also less conspicuous routes in which such compounds enter the terrestrial environment. In contrast to human pharmaceuticals, veterinary medications end up primarily in soil and ground water, the terrestrial ecosystem, contributing relatively less to sediments and surface water. Ecosystem impacts and threats related to health management of livestock in the developing regions of the world exist, although they differ considerably from those of the large-scale swine, poultry and beef operations in the 'west'. Health of animals and people is intricately woven into the classic 'epidemiological triangle' of host (animal, human), agent (infectious, toxic), and environment. Now consider that drugs are exclusively designed to interact physiologically, at cellular and subcellular levels in animals. The powerful companies creating them to benefit the financial health of their investors, plus the health of animals and people, are, from an environmental perspective, "innocent until proven guilty" according to the US Consumer Products Regulations. Not only is this gravely problematic for ecosystem health, but it puts the entire "cost of inaction" squarely onto wildlife, the environment, and those of us working to decreasing environmental costs of anthropogenic activity.

A2.2 DETECTING ECOTOXICOLOGICAL EFFECTS OF PSYCHIATRIC DRUGS BY PREDATOR AVOIDANCE IN THREE-SPINED STICKLEBACK

📅 TUESDAY 4 JULY, 2017 ⌚ 11:10

👤 ERIK HÖGLUND (NORWEGIAN INSTITUTE OF WATER RESEARCH (NIVA), NORWAY), ÅSE ÅTLAND (NORWEGIAN INSTITUTE OF WATER RESEARCH (NIVA), NORWAY)

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Recently, behavioural effects of psychiatric drugs, in concentrations found in municipal waste waters, have been shown in fish. Predator avoidance is closely related to fitness and seems to be regulated with similar hormonal and neural systems as those involved in fear and anxiety in humans. Thus, methods utilizing fear responses in fish have the potential to reveal ecotoxicological effects of pharmaceuticals and other compounds. In this study, we evaluated if behavioural responses to visual predator cues in three-spined stickleback could be applied in high flow throughput ecotoxicological assays. Fish were exposed to citalopram, a selective serotonin reuptake inhibitor, in the concentrations of 1.5 and 15 µg l⁻¹ for 10 and 20 days. After drug exposure, fish were moved to a test arena where individual fish was exposed for two visual predator cues; a shadow from beneath and a passing sea gull silhouette, and an unfamiliar object. The visual predator cues elicited clear responses, with a period of decreased locomotor activity after presentation of the stimulus. Moreover, citalopram exposure was reflected in a dose dependent suppression of the response to the sea gull silhouette, and a similar trend was observed in response to the shadow from beneath. However, there were no drug induced effect in response to the unfamiliar object. This demonstrates that ecotoxicological effects of citalopram in ecologically relevant concentrations can be detected with a high throughput behavioural assay. Moreover, the stimulus specific response emphasizes the need for using ecologically relevant stimuli for detecting ecotoxicological effects of psychiatric drugs.

A2.3 INTRASPECIFIC VARIATION IN TOLERANCE TO PSYCHOACTIVE PHARMACEUTICALS IN ZEBRAFISH (*DANIO RERIO*)

📅 TUESDAY 4 JULY, 2017 ⌚ 11:25

👤 LAURA VOSSEN (UPPSALA UNIVERSITY, SWEDEN), OLY SEN SARMA (UPPSALA UNIVERSITY, SWEDEN), FREDRIK JUTFELT (NORWEGIAN UNIVERSITY FOR SCIENCE AND TECHNOLOGY, NORWAY), TOMAS BRODIN (UMEÅ UNIVERSITY, SWEDEN), JERKER FICK (UMEÅ UNIVERSITY, SWEDEN), SVANTE WINBERG (UPPSALA UNIVERSITY, SWEDEN)

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Environmental pollution by pharmaceuticals is increasingly recognized as a major threat to aquatic ecosystems worldwide. Amongst the most prescribed pharmaceuticals globally are the benzodiazepines (e.g. Valium), a class of psychoactive drugs used to treat anxiety and induce sedation. Benzodiazepines are highly stable in the environment, and their target, the GABA_A receptor, is evolutionarily conserved throughout the vertebrates. Behavioural changes have been described for juvenile Eurasian perch (*Perca fluviatilis*) and Fathead minnows (*Pimephales promelas*) at environmental concentrations. Also zebrafish (*Danio rerio*) show increased activity and decreased anxiety after acute exposure to the benzodiazepine oxazepam. Interestingly, our initial long-term exposure experiments suggest that zebrafish display considerable intraspecific variation in oxazepam tolerance. Provided this tolerance has a genetic basis, adaptation from standing genetic variation could in principle maintain fish populations. Here we analyse the physiological and genetic basis of oxazepam tolerance, including cortisol measurements, concentrations of monoamine neurotransmitters as well as mRNA expression of the GABA_A receptor subunits and enzymes involved in the metabolism of oxazepam. We then correlate the physiological and genetic measurements with a behavioural measure of oxazepam tolerance. The results will shed light on the potential for intraspecific variation to mitigate the effects of benzodiazepine pollution.

A2.4 ECOLOGICAL EFFECTS OF PHARMACEUTICALS IN THE ENVIRONMENT – FROM LAB EXPERIMENTS TO FIELD STUDIES

📅 TUESDAY 4 JULY, 2017 ⌚ 11:40

👤 TOMAS BRODIN (UMEÅ UNIVERSITY, SWEDEN), JONATAN KLAMINDER (UMEÅ UNIVERSITY, SWEDEN), GUSTAV HELLSTRÖM (SLU, SWEDEN), ANNELIE LAGESSON (UMEÅ UNIVERSITY, SWEDEN), MICAEL JONSSON (UMEÅ UNIVERSITY, SWEDEN), JERKER FICK (UMEÅ UNIVERSITY, SWEDEN)

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Humans consume more pharmaceuticals than ever and consumption is set to rise. As a consequence, increasing amounts of pharmaceuticals are released into waterways worldwide with virtually no knowledge of how they might affect aquatic ecosystems. Some conspicuous effects of these emerging contaminants are already evident including the feminization of fish by contraceptive residue. However, recent work suggests that important effects of pharmaceuticals in aquatic environments are much more widespread than currently believed, and that these effects may result in major changes in species interactions, population survival and ecosystem functioning. In several earlier laboratory studies, we have shown that concentrations of pharmaceuticals presently found in waterways alter important behavioural traits in both aquatic macroinvertebrates and fish, and that this in turn affects both feeding efficiency and predation risk. These results suggest that pharmaceutical contamination of aquatic environments may change species interactions, in particular predator-prey interactions, with severe ecosystem effects as potential consequence. Recently our research focus has turned towards realistic large-scale studies in lakes and rivers using acoustic telemetry to test if findings from the lab also hold in natural settings. The overall finding of the studies suggests that effects of pharmaceutical contamination of natural systems might be much more widespread than we predict based on conventional ecotoxicological tests. Our results highlight the importance of validating lab-results with field-studies, since the increased complexity of real ecosystems can produce unexpected effects of contamination.

A2.5 THE USE OF AN *IN VITRO* FISH GILL MODEL TO BETTER UNDERSTAND THE FACTORS THAT INFLUENCE FRESHWATER PHARMACEUTICAL UPTAKE

TUESDAY 4 JULY, 2017 12:10

ELISABETH D CHANG (KING'S COLLEGE LONDON, UNITED KINGDOM), STEWART OWEN (ASTRAZENECA, UNITED KINGDOM), CHRISTER HOGSTRAND (KING'S COLLEGE LONDON, UNITED KINGDOM), NIC BURY (UNIVERSITY OF SUFFOLK, UNITED KINGDOM)

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Pharmaceuticals are becoming increasingly prevalent in the environment. To enable policy makers, regulators and industry to prioritise compound, or classes of compounds, that may require a management strategy it is necessary to identify those pharmaceuticals that pose a potential environmental risk. A component of risk evaluation requires studies using fish with bioaccumulation forming part of this assessment. There is a move to find alternatives to whole animal testing as part of the 3Rs (refinement, reduction and replacement) agenda. Current *in silico* models of organic compound uptake in fish are based primarily on lipophilic, neutral compounds; these models may not be applicable to polar or ionisable compounds. It is estimated that 70% of pharmaceuticals are ionisable compounds and the form they take is dependent on their acid dissociation constant (pKa) and pH of the surrounding water. Empirical data is necessary to develop models of uptake of ionisable compounds. A primary fish gill cell culture system has shown significant promise as an *in vitro* replacement model system for whole fish compound uptake studies as well as environmental monitoring. The current presentation will present some of this data with a specific focus on the relationship between the uptake of the pharmaceutical and various chemical parameters such as Log K_{ow}, Log S, Log D, and pKa.

A2.6 NOT ONLY FISH - INVERTEBRATES IN PHARMACEUTICAL ECOTOXICOLOGY

TUESDAY 4 JULY, 2017 12:25

JOHAN FAHLMAN (DEPARTMENT OF ECOLOGY AND ENVIRONMENTAL SCIENCE, UMU, SWEDEN), JERKER FICK (DEPARTMENT OF CHEMISTRY, UMU, SWEDEN), MICAEL JONSSON (DEPARTMENT OF ECOLOGY AND ENVIRONMENTAL SCIENCE, UMU, SWEDEN), TOMAS BRODIN (DEPARTMENT OF ECOLOGY AND ENVIRONMENTAL SCIENCE, UMU, SWEDEN), JOHAN LIDMAN (DEPARTMENT OF ECOLOGY AND ENVIRONMENTAL SCIENCE, UMU, SWEDEN), JONATAN KLAMINDER (DEPARTMENT OF ECOLOGY AND ENVIRONMENTAL SCIENCE, UMU, SWEDEN)

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The majority of all animals are invertebrates. Still, our knowledge about uptake and effects of pharmaceuticals in invertebrates living in contaminated ecosystems is limited. This presentation synthesizes our recent findings from: 1) field monitoring assays of wild insects living in contaminated environments; 2) field manipulation experiments; and 3) behavioural effect studies. Our analyses of aquatic and terrestrial invertebrates (N=342) living in, or adjacent to, effluent contaminated streams in Australia

reveal that more than 60 different types of active pharmaceutical compounds are occurring in detectable concentrations in their tissues, sometime even in 'pristine' systems. In this assay we also found that surprisingly high tissue concentrations were more than 10 of the analysed drugs occur in concentrations above 1 µg/g. In a controlled whole ecosystem manipulation experiment, we found that the food-web transfer of pharmaceuticals - a route of exposure that is difficult to mimic in artificial laboratory experiments - can be a substantial source of drugs and induce increasing tissue concentrations in invertebrates despite large reductions in water concentrations. Our laboratory effect studies indicate that behavioural and developmental effects can arise among insects (Zygoptera and Chironomidae, respectively) when tissue concentrations of some drugs (i.e. Fexofenadine) comparable to field conditions are generated in laboratory environments. Moreover, these latter tests also reveal some unexplained effects on important ecosystem processes involving nutrient (carbon and nitrogen) cycling. The latter motivates our planned whole lake experiments.

A2.7 LEARNED LESSONS FROM WILDLIFE TOXICOLOGY TO IMPROVE THE RISK ASSESSMENT OF PHARMACEUTICALS

TUESDAY 4 JULY, 2017 13:40

RAFAEL MATEO (UNIVERSITY OF CASTILLA-LA MANCHA, SPAIN)

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The severe impact of diclofenac on populations of Asian vultures has emphasized the need of improving the risk assessment of veterinary drugs. Despite this, diclofenac has been recently approved for veterinary practice in Europe ignoring that in some regions livestock carcasses are consumed by avian scavengers. Ongoing studies confirm the risk of exposure to diclofenac and other non-steroidal anti-inflammatory drugs like flunixin in European vultures. Other veterinary treatments such as topical antiparasitics are a significant source of exposure to neurotoxic compounds in the endangered bearded vulture in Spain. As much as 71% of lamb feet collected at slaughterhouses and used to feed bearded vultures at feeding stations contain residues of antiparasitics, especially diazinon. Risk assessment of this anticholinesterase compound reveals the possibility of adverse effects on thermoregulation that can be relevant in a species that breeds at high altitudes. Exposure to antibiotics in vultures in the Iberian Peninsula has been studied and fluoroquinolones and other families of antibiotics have been detected in 15% of plasma samples of griffon vultures. However, further research is required to assess their effects on avian scavengers. Finally, birds scavenging on carcasses of animals euthanized with barbiturates also become poisoned. A rigorous risk assessment of veterinary drugs must take into account the possibility that carcasses of treated animals are consumed by wildlife. The use of alternatives less toxic for scavengers and the implementation of other measures to reduce the risk of exposure will require the active involvement of veterinary practitioners.

A2.8 *IN SILICO* APPROACHES TO PREDICTING THE EFFECTS OF PHARMACEUTICALS ON ENVIRONMENTAL SPECIES

TUESDAY 4 JULY, 2017 14:20

JUDITH C MADDEN (LIVERPOOL JOHN MOORES UNIVERSITY, UNITED KINGDOM), CLAIRE M ELLISON (LIVERPOOL JOHN MOORES UNIVERSITY, UNITED KINGDOM), MARK TD CRONIN (LIVERPOOL JOHN MOORES UNIVERSITY, UNITED KINGDOM)

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Improvements in analytical methods have led to greater awareness, and concern, regarding active pharmaceutical ingredients (APIs) in the environment. Due to a scarcity of consistent data, relatively little is known about potential effects of these compounds on environmental species. Development of predictive *in silico* tools is therefore a key requirement for estimating potential environmental effects for those APIs with data gaps. Whilst many predictive models for environmental toxicity have been published, these have predominantly been developed for industrial compounds, hence their applicability to APIs needs to be established or new models developed covering appropriate chemical space. Differences between the two groups of chemicals require consideration, for example, APIs are designed to be pharmacologically active and the majority are ionisable whereas the converse is true for industrial compounds. An important initiative in this area is the EU IMI iPIE project (a collaboration of pharmaceutical industries, SMEs and academia) that aims to develop a framework to support intelligent testing of APIs in development and to prioritise legacy APIs for environmental risk assessment and/or (bio)monitoring. Recent efforts in the development of *in silico* models for predicting acute toxicity to environmental species, focussing on their applicability domain in relation to APIs, are discussed.

A2.9 REVERSIBLE BEHAVIOURAL ALTERATIONS IN BURBOT, *LOTA LOTA*, FROM EXPOSURE TO THE ANXIOLYTIC DRUG OXAZEPAM

TUESDAY 4 JULY, 2017 14:50

JOSEFIN SUNDIN (UPPSALA UNIVERSITY, SWEDEN), FREDRIK JUTFELT (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY), JERKER FICK (UMEÅ UNIVERSITY, SWEDEN), MAGNUS THORLACIUS (UMEÅ UNIVERSITY, SWEDEN), TOMAS BRODIN (UMEÅ UNIVERSITY, SWEDEN)

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One group of pharmaceuticals that are frequently detected in the environment are anxiolytic drugs, i.e., pharmaceuticals used to treat anxiety, such as antidepressants. The most commonly used anxiolytic drugs globally are benzodiazepines. Benzodiazepines persist in wastewater effluent and can be found at high concentrations in treated effluent. Further, several benzodiazepines are resistant to photodegradation, enabling them to persist in the environment. Benzodiazepines are designed to alter human behaviour by binding to GABA-receptors, which are found in a wide range of animals including all vertebrates. We investigated the effect

of the benzodiazepine oxazepam on behaviour using the burbot, *Lota lota*. We found that swimming activity, diurnal as well as nocturnal, was affected by oxazepam. There was also an effect on boldness, with fish exposed to high levels of oxazepam spending more time hiding than the control fish. Interestingly, the effects of oxazepam were no longer detectible when the fish were tested again after being kept in water without drugs for five days. Our results suggest that effects of pharmaceuticals may be reversible, if the exposure duration is relatively short and the animal has the possibility to move to clean water.

A2.10 SEX, STRESS AND FOOD: IMPACTS OF ANTIDEPRESSANTS IN THE ENVIRONMENT ON BIRDS

TUESDAY 4 JULY, 2017 16:00

KATHRYN ARNOLD (UNIVERSITY OF YORK)

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Birds are exposed to human pharmaceuticals when they forage on invertebrates at sewage treatment plants and on sewage fertilised fields, but the impacts remain largely unknown. Antidepressants are both heavily prescribed and persistent in the environment. Consequently, we experimentally investigated the effects of environmentally relevant doses of the antidepressant fluoxetine (Prozac) on starling *Sturnus vulgaris* courtship traits. Chronic exposure to low concentrations of fluoxetine altered the rate of development of breeding colouration in females. In mate choice trials, exposure to fluoxetine did not seem to alter male attractiveness, but reduced female attractiveness. Appetite and thermoregulation of fluoxetine treated birds were also significantly negatively affected compared with controls. Hormonal and behavioural mechanisms for these effects were then explored. Our results appear to mirror common side effects of fluoxetine in humans namely sexual dysfunction, vasoconstriction and appetite changes. If such disruption to female courtship traits and behaviours, for example, observed here were to occur in wild birds then it could reduce breeding success and contribute to observed population declines.

A2.11 PREDICTING THE PERSISTENCE OF PHARMACEUTICALS IN COMPLEX AQUATIC ECOSYSTEMS

TUESDAY 4 JULY, 2017 16:30

JONATAN KLAMINDER (UMEÅ UNIVERSITY, SWEDEN)

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The incomplete removal of pharmaceuticals from wastewater plants was first reported more than 50 years ago. Since then an increasing number of studies have revealed the presence of excreted pharmaceutical in surface waters - especially after the 1990s when new techniques facilitated detection of these contaminants in complex environmental matrices. Persistence of individual pharmaceuticals is a key property when doing environmental risk assessments. But how representative are simplified laboratory experiments for estimating dissipation rates in more complex and natural ecosystems? This presentation will discuss environmental processes, often lacking in laboratory environments, which can explain why common methodological approaches sometimes greatly underestimate the persistence of active pharmaceutical substances in natural aquatic environments. I will also explain why I think current research on the persistence of pharmaceuticals in such environments is lagging far behind other fields within ecotoxicology.

A2.12 ENVIRONMENTAL RISK ASSESSMENT OF ACTIVE PHARMACEUTICAL INGREDIENTS USED IN HUMAN MEDICINAL PRODUCTS: EUROPE-WIDE VARIATION IN RISK QUOTIENT

TUESDAY 4 JULY, 2017 POSTER SESSION

STEWART OWEN (ASTRAZENECA, UNITED KINGDOM), LINA GUNNARSSON (UNIVERSITY OF EXETER, UNITED KINGDOM), JASON SNAPE (ASTRAZENECA, UNITED KINGDOM), KATHY HUTCHINSON (ASTRAZENECA, UNITED KINGDOM), BAS VERBRUGGEN (UNIVERSITY OF EXETER, UNITED KINGDOM), DEAN LEVERETT (WCA ENVIRONMENT, UNITED KINGDOM), BECKY MARKS (WCA ENVIRONMENT, UNITED KINGDOM), CHARLES TYLER (UNIVERSITY OF EXETER, UNITED KINGDOM)

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Pharmaceuticals undergo regulatory environmental risk assessments (ERAs) that estimate the potential environmental impact on a product-by-product basis rather than a substance-by-substance basis. In the cases where an active pharmaceutical ingredient (API), or substance, is used to treat multiple clinical diseases, there is the potential to under-estimate environmental impact. Here we determine the total substance or API risk. We have: 1) Identified and collected published no observed effect concentrations (NOECs) for available APIs (excluding anti-infectives and anti-parasitic products); 2) Collated human consumption data for each of these APIs in European Countries where these products are licenced for use; 3) Conducted a worst case exposure assessment (predicted environmental concentration; PEC) and estimated the total risk posed by each API; 4) Analysed the variability in the risk quotients (RQs) for each API across Europe.

Using a total PEC based approach, we determine that for most APIs (>95%) they pose low or insignificant environmental risk based on the standard ecotoxicology data available. The highest RQs are associated with endocrine active APIs that have high potency - e.g. ethinyloestradiol. We also show RQs for the same API can be highly variable between European countries.

A2.13 FISH ON STEROIDS: HOW DOES 17B-TRENBOLONE AFFECT NON-REPRODUCTIVE BEHAVIOR IN MOSQUITO FISH?

TUESDAY 4 JULY, 2017 POSTER SESSION

ANNELIE LAGESSON (DEPARTMENT OF ECOLOGY AND ENVIRONMENTAL SCIENCE, UMEÅ UNIVERSITY, SWEDEN), MINNA SAARISTO (SCHOOL OF BIOLOGICAL SCIENCES, MONASH UNIVERSITY, AUSTRALIA), TOMAS BRODIN (DEPARTMENT OF ECOLOGY AND ENVIRONMENTAL SCIENCE, SWEDEN), JERKER FICK (DEPARTMENT OF CHEMISTRY, UMEÅ UNIVERSITY, SWEDEN), JAKE MARTIN (SCHOOL OF BIOLOGICAL SCIENCES, MONASH UNIVERSITY, AUSTRALIA), JONATAN KLAMINDER (DEPARTMENT OF ECOLOGY AND ENVIRONMENTAL SCIENCE, SWEDEN), BOB WONG (SCHOOL OF BIOLOGICAL SCIENCES, MONASH UNIVERSITY, AUSTRALIA)

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Trenbolone acetate, an endocrine disrupting chemical (EDC), is a heavily used growth promoter in agriculture (beef production) in many parts of the world, among them USA and Australia. Its metabolite 17b-trenbolone (hereafter referred to as trenbolone) is extremely stable in animal waste and enters aquatic environments through direct discharge of livestock urine and manure or runoff from feedlots. Studies have shown that trenbolone can affect morphological and physiological endpoints in fish and also disrupt reproductive behaviours. So far however, little is known about how it may affect other types of behaviour in fish. The aim of this study was to test how short-term (21-day) exposure to an environmentally relevant concentration of trenbolone (10 ng/L) affects anti-predator behaviour, activity and exploration behaviour of the eastern mosquitofish (*Gambusia holbrooki*) in two different temperatures, 20 and 30°C. The anti-predator behaviour was assayed by subjecting the fish to a simulated predator strike, and the activity and exploration behaviour were assayed in a maze trial. Results of trenbolone exposure, temperature and their interaction on behavioural endpoints will be presented. In addition, tissue samples were taken over the course of the exposure period (12h, 16h, 24h, 48h, 3d-7d, 11d, 20d) to explore bioconcentration of trenbolone.

A3 CLIMATE CHANGE AND AQUATIC LIFE: EFFECTS OF MULTIPLE DRIVERS, FROM MOLECULES TO POPULATIONS

ORGANISED BY: LUCY TURNER (PLYMOUTH UNIVERSITY, UK)
AND MANUELA TRUEBANO GARCIA (PLYMOUTH UNIVERSITY, UK)

A3.1 INTRASPECIFIC VARIATION IN THERMAL TOLERANCE, HYPOXIA TOLERANCE, AND METABOLIC RATE: IMPLICATIONS FOR ORGANISMAL RESPONSES TO CLIMATE CHANGE

TUESDAY 4 JULY, 2017 **10:30**

PATRICIA M SCHULTE (UNIVERSITY OF BRITISH COLUMBIA, CANADA), **TIMOTHY M HEALY** (UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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Functional and genetic associations among traits can influence a species' capacity for adaptation to environmental change. For aquatic organisms, upper thermal tolerance and hypoxia tolerance are traits that may affect sensitivity to climate change, and theory predicts that these traits may be positively associated. To test this prediction, we assessed the thermal and hypoxia tolerances of ~1,200 Atlantic killifish, *Fundulus heteroclitus*, from populations across intraspecific contact zones between two subspecies that differ in critical thermal maxima (CT_{max}), metabolic rate, and time to loss of equilibrium in hypoxia (LOE_{hyp}). Consistent with the possibility of a functional association between thermal and hypoxia tolerance, the more thermally tolerant subspecies is also more tolerant of hypoxia, and has a lower metabolic rate. However, among populations thermal tolerance varies linearly with latitude whereas hypoxia tolerance exhibits steep phenotypic breaks across both contact zones, and among contact zone individuals CT_{max} and LOE_{hyp} are not correlated. These results suggest that CT_{max} and LOE_{hyp} are not functionally associated in this species, and that any genetic bases for variation in these traits are likely independent. As a result, rates of adaptive change in these traits as a result of climate change will likely neither be expedited nor be hindered due to trait associations.

A3.2 THE ROLE OF REVERSIBLE PLASTICITY UNDER TEMPERATURE AND PH STRESS IN LOCALLY ADAPTED *PHYLLAPLYSIA TAYLORI* POPULATIONS

TUESDAY 4 JULY, 2017 **11:10**

RICHELLE L TANNER (UNIVERSITY OF CALIFORNIA BERKELEY, UNITED STATES), **JONATHAN H STILLMAN** (UNIVERSITY OF CALIFORNIA BERKELEY, UNITED STATES)

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Nearshore eelgrass habitats along the Pacific coast of North America play an important role in erosion control, fish and invertebrate development, and localized ocean acidification buffering. A key grazer in these intertidal ecosystems is *Phyllaplysia taylori*, a sea hare, living on the *Zostera marina* eelgrass blades, that feeds on epiphytic algae, diatoms, and bryozoans, keeping blades clean for increased photosynthesis and growth. *P. taylori* have direct development, evident in ecological surveys indicating limited dispersal potential that could lead to reduced gene flow between populations. Physiological measures of metabolic rate, critical thermal maximum, and acclimation response ratio were determined across populations with no evident pattern between acclimation temperatures or before/after a heat shock. Evidence of local adaptation in thermal plasticity traits is strong, as the population characteristics measured did not scale latitudinally or with ecological and environmental characteristics. These populations are subject to fluctuations in temperature and pH in nature, which were examined in the laboratory in one population under three simulated regimes: stress (future conditions), ambient (current), and constant. The effects of pH as an added stressor were investigated under the same physiological measures of metabolic rate as above to look for additive or synergistic effects. Performance curves were estimated to further understand how reversible plasticity plays a role in these populations' differences in thermal tolerance under multiple stressors. Since *P. taylori* plays a large role in maintaining eelgrass health, understanding these local differences in plasticity will have significant implications for promoting eelgrass restoration efforts under future climatic regimes.

A3.3 TEMPERATURE AND UV-B RADIATION: INTERACTIVE EFFECTS ON SURVIVAL, GROWTH AND DNA REPAIR MECHANISMS

TUESDAY 4 JULY, 2017 11:25

CRAIG E FRANKLIN (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA), SAMUEL MORISON (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA), REBECCA L CRAMP (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA), LESLEY A ALTON (MONASH UNIVERSITY, AUSTRALIA)

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Amphibians are at the forefront of a global biodiversity crisis. Of concern are population declines that have occurred in areas with little or no discernible human interference. These near-pristine areas have included montane regions in Australia and Central America. Ultraviolet-B radiation (UVBR) has been implicated in these declines and may have resulted from a synergistic interaction between higher UV levels and cooler temperatures that occur at higher altitudes. Attempting to provide a mechanistic explanation for amphibian declines at high altitudes requires a greater understanding of UVBR and its impacts on amphibian physiology. UV-B radiation can affect DNA structure resulting in the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4 PPs) which create lesions in the DNA strands that interrupt replication and can cause mutations or cell apoptosis. To repair this DNA damage, most organisms utilise DNA repair mechanisms, the most efficient of which is enzymatic photoreactivation. Nucleotide excision repair (NER) is also utilised to repair DNA damage, though is more energetically costly. In this study we examined the effects of temperatures on the UVBR-associated DNA damage, DNA repair rates, photolyase gene expression. We hypothesised that individuals exposed to UVBR at lower temperatures would have slower repair rates than individuals exposed at higher temperatures, resulting in accumulated damage and effects of growth and survival.

A3.4 IMPORTANCE OF FRAMING CLIMATE CHANGE BIOLOGY IN AN ECOLOGICALLY RELEVANT CONTEXT: INSIGHTS FROM THE ROCKY INTERTIDAL

TUESDAY 4 JULY, 2017 11:40

ANNE E TODGHAM (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES)

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Our capacity to predict the effects of global change on marine ecosystems requires that we consider the complexity of current and future environmental conditions. Single stressor experiments commonly fail to accurately capture the tolerance of species to environmental change as organisms live in complex, multivariate environments. In addition to examining how organisms respond to multiple co-occurring stressors, other aspects of environmental change such as timing (e.g. serial exposures, duration) and variability (e.g. predictable vs. stochastic, magnitude) may be important components of environmental complexity structuring an organism's response to change. In this talk I will provide examples of how incorporating environmental complexity in intertidal physiology research has broadened our understanding of stress tolerance mechanisms in limpets. The hope is to initiate discussions of how we, as a research community, can best design multiple stressor experiments that are most relevant to our species of interest but also allow for comparative analyses of resilience to change.

A3.5 CONSEQUENCES OF OCEAN ACIDIFICATION COMBINED WITH HYPOXIA OR MANGANESE ON DIFFERENT LIFE STAGES AND ORGANISATION LEVELS OF THE NORWAY LOBSTER

TUESDAY 4 JULY, 2017 12:10

ANNA-SARA KRÅNG (IVL SWEDISH ENVIRONMENTAL RESEARCH INSTITUTE, SWEDEN), HANNAH K STYF (SWECO ENVIRONMENT AB, SWEDEN), BODIL HERNROTH (KRISTIANSTAD UNIVERSITY, SWEDEN), SUSANNE P BADEN (UNIVERSITY OF GOTHENBURG, SWEDEN), FREDRIK JUTFELT (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY), JONAS MATTSSON (UNIVERSITY OF GOTHENBURG, SWEDEN), SUSANNE P ERIKSSON (UNIVERSITY OF GOTHENBURG, SWEDEN)

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Ocean acidification (OA) is of great concern to marine biota, yet we know very little of the combined effects with other environmental stressors such as spread of oxygen depleted areas. The Norway lobster, *Nephrops norvegicus*, is one of the commercially most important fishery species in Europe. It inhabits coastal soft bottom sediments subjected to periodic hypoxia. Hypoxia in turn infers increased bioavailability of the heavy metal manganese that normally is bound to the sediment. In this comprehensive study, we investigated possible interactions between longer-term exposure to elevated pCO₂ at concentrations postulated by 2100, and more short-term exposure to hypoxia or manganese on different life stages of the Norway lobster. We demonstrate severe impacts at different

organisation levels; from suppressed immune response to bacterial infection, to effects on embryonic heart rate, respiration, foraging behaviour and CO₂ avoidance. Clearly, there are risks of great impact on lobster condition and biomass at these future stress scenarios. However, our results also demonstrate that susceptibility vary greatly between life stages and organisation levels. Nevertheless, the combination of stressors often had the most severe effects, implying future studies on interactions with pollutants and natural variables to better predict the influence OA will have on marine organisms.

A3.6 LINKING PHYSIOLOGICAL EFFECTS OF CLIMATE CHANGE STRESSORS WITH EFFECTIVE CONSERVATION: LESSONS FROM AN ENDANGERED FISH

📅 TUESDAY 4 JULY, 2017 ⌚ 12:25

👤 NANN A FANGUE (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES), LISA M KOMOROSKE (UNIVERSITY OF MASSACHUSETTS AMHERST, UNITED STATES), KEN M JEFFRIES (UNIVERSITY OF MANITOBA, CANADA), RICHARD E CONNON (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES)

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Since the implementation of the US Federal Endangered Species Act (ESA), no listed fish species has gone extinct. However, approximately 80% of freshwater fishes are facing extinction over the next 100 years, and in California, native freshwater and anadromous fishes reflect this trend. For effective species conservation and management, defining suitable habitat for fishes is of fundamental importance, yet translating physiological measures of performance into implementable regulatory numeric criteria remains difficult. In this talk I will highlight a variety of approaches that we have used to understand the physiological and behavioral responses of a California endemic, endangered fish, the Delta Smelt (DS, *Hypomesus transpacificus*). Importantly, the abundance of DS has reached a historic low with a zero abundance index returned in 2015 derived from only a handful of fish collected that year. Our studies have spanned life stage, biological levels of organization, incorporated single and multiple ecologically-relevant stressors, and integrated these data with predictive regional global climate models. Taken together, our recent work demonstrates that the integration of functional and mechanistic studies with ecosystem-based management can be achieved to best inform conservation and restoration strategies. It is the case, however, that extinction is an eventuality for DS unless bold intervening steps are taken, informed by science and motivated by human will. Barriers to recovery will be discussed.

A3.7 PREDICTING EVOLUTIONARY RESPONSES OF REEF FISHES TO CLIMATE CHANGE: PROGRESS AND CHALLENGES

📅 TUESDAY 4 JULY, 2017 ⌚ 13:40

👤 PHILIP MUNDAY (JAMES COOK UNIVERSITY, AUSTRALIA)

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Many short-term experiments have demonstrated the potential impacts of ocean warming and acidification on marine organisms. However, longer-term experiments are needed to test the capacity for acclimation and adaptation to these stressors. In this talk I will describe unique multigenerational experiments being used to test the effects of warming and acidification on coral reef fishes and explore their capacity for acclimation and adaptation. These same experiments are being coupled with modern molecular approaches to understand the mechanisms underlying phenotypic responses to warming and acidification, and the mechanisms by which adaptive responses occur within and between generations. Our results indicate that there is limited capacity for reversible acclimation to elevated temperature in adult reef fishes, but exposure to higher temperatures during early life can induce beneficial developmental plasticity that improves performance at higher temperatures later in life. Parental and multigenerational exposure to elevated temperatures can have further beneficial effects on the performance of fish at higher temperatures, both through transgenerational plasticity and the presence of heritable genetic variation. Nevertheless, there are limits to these beneficial effects and major experimental challenges remain in disentangling various forms of plasticity, the mechanisms by which the environmental conditions experienced in one generation influence the performance of future generations, and the constraints of multiple climate change stressors on adaptive responses.

A3.8 AN INTERPLAY BETWEEN PLASTICITY, EPIGENETICS, AND PARENTAL PHENOTYPE DETERMINES IMPACTS OF OCEAN ACIDIFICATION ON A REEF FISH

TUESDAY 4 JULY, 2017 14:20

CELIA SCHUNTER (KING ABDULLAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, SAUDI ARABIA), MEGAN WELCH (COLLEGE OF MARINE AND ENVIRONMENTAL SCIENCES, JAMES COOK UNIVERSITY, AUSTRALIA), GÖRAN E NILSSON (DEPARTMENT OF BIOSCIENCES, UNIVERSITY OF OSLO, NORWAY), JODIE L RUMMER (ARC CENTRE OF EXCELLENCE FOR CORAL REEF STUDIES, JAMES COOK UNIVERSITY, AUSTRALIA), PHILIP L MUNDAY (ARC CENTRE OF EXCELLENCE FOR CORAL REEF STUDIES, JAMES COOK UNIVERSITY, AUSTRALIA), TIMOTHY RAVASI (KING ABDULLAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, SAUDI ARABIA)

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The impacts of ocean acidification will depend on the ability of marine organisms to tolerate, acclimate, and eventually adapt to changes in ocean chemistry. Here we use a unique transgenerational experiment to show the molecular response of a coral reef fish to near-future CO₂ conditions and determine how short-term, developmental and transgenerational responses to elevated CO₂ may be influenced by variation in behavioural tolerance in the parental phenotype. Within-generational responses in gene expression to end of century predicted CO₂ levels indicate that a self-amplifying circle in GABAergic neurotransmission is triggered explaining neurological and behavioural impairments. Furthermore, epigenetic regulator genes exhibited a within-generation specific response with some divergence due to parental phenotype. Importantly, we find a recovery pattern for the majority of within-generation responses following exposure of parents to high CO₂ conditions. Our results show that parental variation in tolerance and cross-generation exposure to elevated CO₂ are crucial factors in determining the response to changing ocean chemistry.

A3.9 FISH ON ACID – THE ECOPHYSIOLOGICAL CONSEQUENCES OF OCEAN ACIDIFICATION AND WARMING ON FISH

TUESDAY 4 JULY, 2017 14:35

FELIX C MARK (ALFRED WEGENER INSTITUTE, GERMANY), CONSORTIUM 4 MEMBERS BIOACID II (BMBF BIOACID, GERMANY), CONSORTIUM 3 MEMBERS BIOACID III (BMBF BIOACID, GERMANY)

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Within the second and third phase of the German ocean acidification research programme BIOACID (2012-2017) we investigated how the combined effects of ocean acidification and warming (OAW) affect different life stages and interactions between Atlantic and Polar cod and their prey.

Objectives included addressing the question whether OAW affects interacting species differently due to divergent physiological optima and ranges, expressed in thermal tolerance windows and associated performance capacities and phenologies of specific life stages. We aimed to identify fundamental mechanisms by unravelling the connections between levels of biological organisation, from genomic, molecular to cellular, individual and population level. Scopes for acclimation (physiology and behaviour) and adaptation (evolution) that together define species resilience were studied in various life stages (eggs, larvae, juveniles, adults) to identify the most sensitive one(s). Functional determinants of individual fitness such as ion and acid-base regulation, mitochondrial energy metabolism, and immune response, as well as their dependence on food quality and availability were also examined. I will present an overview of our results and how they are implemented into future projections of a changing ocean and identify open questions that still need further research.

A3.15 MULTI-OMICS APPROACHES TO UNDERSTANDING RESPONSES TO CHANGE

TUESDAY 4 JULY, 2017 16:00

MELODY S CLARK (BRITISH ANTARCTIC SURVEY, UNITED KINGDOM)

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Our world is changing and for many reasons we need to be able to predict what our future ecosystems will look like. But how do we even start to tackle such a complex issue? Currently there are a range of different approaches taken including the a posteriori identification of how communities have changed in particular habitats over time correlated with changing environmental conditions and the evaluation of genetic differences between a species or closely related species in differing habitats (often along a latitudinal gradient). Both of these are very useful retrospective evaluations of responses under natural conditions. An alternative and more real-time predictive approach is that of experimental manipulation in the laboratory. This approach is often accused of not being realistic in terms of the rates at which environmental change is applied, but it does allow for carefully controlled conditions to evaluate between species' differences and the cellular mechanisms underlying resilience or sensitivity. It also enables the detailed evaluation of a limited

number of “model” environmental organisms and the extrapolation of these results to the far more numerous non-tractable species. The main question is why do some species fail and others thrive, but also how many species do we need to predict change in an ecosystem? In this talk I will discuss recent results using a range of ‘Omics’ approaches to understand cellular responses to change in marine species, the advantages of this varied approach in non-model species and how these can be used to benefit future biodiversity predictions.

A3.16 PHENOMIC RESPONSES OF AQUATIC EMBRYOS TO ENVIRONMENTAL CHANGE: APPLICATION OF A NOVEL TECHNOLOGY

TUESDAY 4 JULY, 2017 **16:30**

OLIVER TILLS (PLYMOUTH UNIVERSITY, UNITED KINGDOM), JOHN SPICER (PLYMOUTH UNIVERSITY, UNITED KINGDOM), ANDREW GRIMMER (PLYMOUTH UNIVERSITY, UNITED KINGDOM), SIMONE MARINI (INSTITUTE OF MARINE SCIENCE (ISMAR), NATIONAL RESEARCH COUNCIL OF ITALY, ITALY), SIMON RUNDLE (PLYMOUTH UNIVERSITY, UNITED KINGDOM)

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Description of complex phenome-level (an organisms? observable characteristics) responses of developing organisms is inherently challenging, owing both to the dynamic nature of development and the high-dimensionality of trait measurements (rates, timings, distances, durations etc). Such a capability would significantly benefit our understanding of organismal form and function and lead to advances in our interpretation and integration of molecular-level responses. We have developed an automated platform for phenomics in aquatic embryos that combines hardware for time-lapse bioimaging and analytical software for quantification and integration of both physiological and morphological traits. The platform is highly versatile and describes phenomes with high temporal and spatial resolution and in a way that is impractical to do manually. Here, we present: i) how this technology has been used to measure responses of mollusc and crustacean embryos to short- and long-term exposures to elevated temperatures; and ii) the novel insights that this approach has revealed of the complex responses of embryos in terms of their combined morphological and physiological, sub-lethal and lethal, responses. This capability is invaluable for describing the complex responses of organisms to multiple stressors.

A3.17 WILL FISHES BE SMALLER IN A WARMER FUTURE?

TUESDAY 4 JULY, 2017 **16:45**

GÖRAN E NILSSON (UNIVERSITY OF OSLO, NORWAY), SJANNIE LEFEVRE (UNIVERSITY OF OSLO, NORWAY), DAVID J MCKENZIE (UNIVERSITY OF MONTPELLIER, FRANCE)

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In recent years some influential studies projecting the future average body size of fishes have claimed that fish will become smaller when the water temperatures rise. Several of these studies are based on false physiological assumptions regarding the scaling of gills in relation to body mass, as well as the scaling of metabolic rate in fishes. It is remarkable that these assumptions have been widely accepted in fisheries science, since they are easy to disprove both theoretically and by examining available data (Lefevre et al., 2017). There are also claims that Bergmann's rule (originally postulating that endothermic animals become larger in colder areas to compensate for the heat loss to the environment) also applies to fish, without a plausible mechanism. In fact, the largest sharks, rays and teleosts occur in tropical waters. Some studies of body size in fish populations over recent years have argued that fishes are indeed becoming smaller due to global warming, but the interpretation of the data is complicated by issues such as fishing induced selection favouring smaller body sizes. So, if there are no theoretical or empirical evidence for the existence of physiological mechanisms that will bring down the body size of fishes as temperatures rise, are there any reasons to believe that fishes will be smaller in a warmer future?

A3.10 RELATIONSHIP BETWEEN THERMAL TOLERANCE AND HYPOXIA TOLERANCE IN AMAZONIAN FISHES

TUESDAY 4 JULY, 2017 **POSTER SESSION**

ELLEN H JUNG (UNIVERSITY OF BRITISH COLUMBIA, CANADA), ADALBERTO L VAL (BRAZILIAN NATIONAL INSTITUTE FOR RESEARCH OF THE AMAZON, BRAZIL), COLIN J BRAUNER (UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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The Amazon contains 20% of the world's freshwater fish species that are predicted to experience an increase in temperature by up to 4°C within the next century. Increase in temperature will likely be associated with an increase in the frequency, duration, and magnitude of hypoxic events, creating an even greater challenge. However, little is known about the influence of these combined factors as well as the effect of acclimation to elevated temperature on this relationship, which is important to understand in order to accurately predict the potential impact of climate change. To address this, we conducted acute thermal tolerance (CT_{Max}) and hypoxia tolerance (air saturation at loss of equilibrium) assays in 21 species that spanned a broad phylogenetic range. We also investigated the effect of acute and chronic temperature increases on those parameters. In fish at ambient temperature (31°C), we found a positive relationship between the two, where fish that had greater upper thermal limit were more tolerant to severe hypoxia. In fish acutely transferred to 33 or 35°C, there was a reduction in hypoxia tolerance, while acclimation to those same temperatures

partially mitigated that effect. In addition, acclimation significantly increased their CT_{Max}, however most species failed to survive in the highest treatment of 35°C. Hyperoxia (a natural occurrence in the day when temperature is highest) increased acute thermal tolerance. Overall, results indicate that a fairly simple assay may be used to identify species at risk in the changing environment of the Amazon. (Supported by CNPq/FAPEAMINCTADAPTA)

A3.11 OCEAN ACIDIFICATION ALTERS PREDATOR AND PREY BEHAVIOUR IN INVERTEBRATES: JUMPING SNAILS, TROPHIC INTERACTIONS AND NEUROTRANSMITTERS

TUESDAY 4 JULY, 2017 POSTER SESSION

SUE-ANN WATSON (JAMES COOK UNIVERSITY, AUSTRALIA), SJANNIE LEFEVRE (UNIVERSITY OF OSLO, NORWAY), JENNIFER B FIELDS (PITZER COLLEGE, UNITED STATES), PAOLO DOMENICI (ISTITUTO PER L'AMBIENTE MARINO COSTIERO, ITALY), GÖRAN E NILSSON (UNIVERSITY OF OSLO, NORWAY), PHILIP L MUNDAY (JAMES COOK UNIVERSITY, AUSTRALIA)

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Ocean acidification poses a range of threats to marine invertebrates including reduced growth and calcification. However, the potential effects of rising carbon dioxide (CO₂) on marine invertebrate behaviour are largely unknown. Marine conch snails have a modified foot and operculum allowing them to leap backwards rapidly when faced with a predator, such as a venomous cone snail. We habituated humpbacked conch prey and cone snail predator to control (~400 μatm) and elevated (~970 μatm) seawater CO₂ during two studies at Lizard Island Research Station on the Great Barrier Reef and examined their behaviours. We show that projected end-of-century CO₂ levels impair key behaviours of marine invertebrates, including behaviours involved in predator-prey interactions. Elevated-CO₂ halved the number of prey snails that jumped from the predator, increased their latency to jump and altered their escape trajectory. Physical ability to jump was not affected by elevated-CO₂ indicating instead that decision making was impaired. In predators, elevated-CO₂ increased activity more than threefold, but reduced predation rate. Prey snails were treated with gabazine, a GABA receptor antagonist of vertebrate and some invertebrate nervous systems. Gabazine treatment fully restored normal behaviour, indicating a potential interference of neurotransmitter receptor function by elevated-CO₂ in invertebrates, as previously observed in marine fishes. The alteration of fundamental predation and escape behaviours of invertebrates and fishes at projected near-future CO₂ levels could disrupt species interactions, community structure and food web stability with potentially far-reaching implications for marine ecosystems.

A3.12 SURVIVAL AND SALINITY TOLERANCE LIMITS IN THE SNAIL *THEODOXUS FLUVIATILIS*: FRESHWATER VS. BRACKISH WATER LINEAGES

TUESDAY 4 JULY, 2017 POSTER SESSION

AMANDA A WIESENTHAL (ERNST MORITZ ARNDT- UNIVERSITY GREIFSWALD, GERMANY), CHRISTIAN MÜLLER (ERNST MORITZ ARNDT-UNIVERSITY GREIFSWALD, GERMANY), JAN-PETER HILDEBRANDT (ERNST MORITZ ARNDT-UNIVERSITY GREIFSWALD, GERMANY)

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It is commonly accepted that there are morphological and physiological differences between species of the genus *Theodoxus* (Gastropoda: Neritidae) but when it comes to differences within the widely distributed species *T. fluviatilis* there are still open questions. This species has formed regional subgroups (lineages) in northern Germany that appear separate in either fresh or brackish water, yet, according to literature, are indistinguishable by morphology, anatomy or mtRNA markers. Tolerance towards challenging salinities in animals from these lineages, however, is distinct. The question, whether the observed difference in salinity tolerance is a newly developed trait or a derived feature from freshwater individuals that has shifted due to local adaptation after recolonisation of the Baltic sea, remains. In this study physiological limits to salinity stress and range shift abilities were compared between individuals from fresh- and brackish water lineages in a 20-day common garden experiment and acclimation regime. Results showed that the lineages differ in their tolerance towards high and low salinity levels. Brackish water animals struggled in freshwater but performed much better than freshwater individuals when directly transferred into high salinities (20‰). After slowly acclimatising the animals to their challenging salinities, both lineages achieved a range shift in reaction norms and were able to survive salinity levels otherwise lethal to them. Even with this shift freshwater animals were not able to tolerate salinities as high as brackish water animals could. These results indicate that phenotypic plasticity as well as genetic adaptation may determine osmotolerance in the lineages.

A3.13 PHYSIOLOGICAL AND LIFE HISTORY CHALLENGES IN A CHANGING OCEAN: WHAT MULTIGENERATIONAL EXPERIMENTS CAN REVEAL FOR MARINE METAZOANS

TUESDAY 4 JULY, 2017 POSTER SESSION

GLORIA MASSAMBA N'SIALA (UNIVERSITÉ DU QUÉBEC À RIMOUSKI, CANADA), EMMA M GIBBIN (ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE, SWITZERLAND), LEELA J CHAKRAVARTI (JAMES COOK UNIVERSITY, AUSTRALIA), MICHAEL D JARROLD (JAMES COOK UNIVERSITY, AUSTRALIA), FELIX CHRISTEN (UNIVERSITÉ DU QUÉBEC À RIMOUSKI, CANADA), PIERRE BLIER (UNIVERSITÉ DU QUÉBEC À RIMOUSKI, CANADA), PIERO CALOSI (UNIVERSITÉ DU QUÉBEC À RIMOUSKI, CANADA)

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Ocean warming and acidification are concomitant climate drivers that are currently threatening the survival of marine species. How species will respond to these changes depends on their phenotypic plasticity and their potential for adaptation. Little is known about the mechanisms that govern these interactions or how global change will influence these relationships across multiple generations. To test this, we exposed the model marine polychaete *Ophryotrocha labronica* to conditions simulating ocean warming and acidification, in isolation and in combination over five generations to identify: (i) how multiple versus single climate change drivers alter the shape of the relationship between life history and physiology; (ii) the mechanistic link between life-history and physiology; (iii) whether changes in phenotypes are plastic or adaptive. Two juvenile (growth rate; survival to sexual maturity) and two adult (mean reproductive body size; fecundity) life-history traits were measured in each generation, as well as three physiological (cellular ROS content, mitochondrial density; mitochondrial capacity) traits. We found that exposure to warming alone caused increases in: juvenile growth, ROS and mitochondrial density, and decreases in: mean reproductive body size, fecundity and fluctuations in mitochondrial capacity, relative to control conditions. While exposure to ocean acidification alone had only minor effects on juvenile growth. Remarkably, single stressor represented a bigger challenge than when both stressors were present, suggesting that ocean warming and acidification act as opposing vectors of stress to enhance this species' potential for acclimation across multiple generations.

A3.14 DAILY CYCLIC HYPOXIA IMPROVES PALAEMON VARIANS' SURVIVAL WHEN EXPOSED TO ACUTE COPPER TOXICITY AND WHEN EXPOSED TO THERMAL STRESS

TUESDAY 4 JULY, 2017 POSTER SESSION

LUCA PERUZZA (NATIONAL OCEANOGRAPHY CENTRE SOUTHAMPTON, UNITED KINGDOM), ALASTAIR BROWN (NATIONAL OCEANOGRAPHY CENTRE SOUTHAMPTON, UNITED KINGDOM), SVEN THATJE (NATIONAL OCEANOGRAPHY CENTRE SOUTHAMPTON, UNITED KINGDOM), CHRIS HAUTON (NATIONAL OCEANOGRAPHY CENTRE SOUTHAMPTON, UNITED KINGDOM)

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Shallow-water environments are generally characterized by high species number and great productivity. These same habitats are also more vulnerable to climate change (i.e. water temperature, hypoxia) and pollution (i.e. pesticides, industrial wastes). The molecular, physiological and ecological effects of single stressors exposures have been widely studied on numerous species and taxa, but the effects of multiple drivers are still not well characterized and have previously shown complex and unexpected interactive effects. To determine the ecophysiological implications of hypoxia, coupled with temperature or copper, on shallow-water crustaceans, we initially performed a 28-day experiment by mimicking, on a daily base, oxygen fluctuations down to the critical oxygen partial pressure (p_{crit}) for our model species, the ditch shrimp, *Palaemon varians*. Subsequently animals were challenged either for critical thermal maximum (CT_{max}) or for acute copper toxicity (5 and 30 mg Cu^{2+} /L). The interaction of hypoxia with temperature or copper altered the expression of key genes involved in shock response and detoxification. Survival rate was also affected, with unexpected results: CT_{max} was significantly higher in animals that experienced hypoxia, in comparison to controls (t-test, p-value=0.015). Animals kept in hypoxia and subsequently exposed to 30 mg Cu^{2+} /L had a significantly higher survival rate than animals exposed to copper but not to hypoxia (Log-rank test, p-value=0.04). Cyclic hypoxia induces morphological changes in the gills in *P. varians*, by increasing lamellar's length and surface area. Hence, those changes likely enhance animal's survival to thermal stress and toxicants and are identified as an antagonistic effects between hypoxia and copper pollution.

A3.18 NEAR-FUTURE CARBON DIOXIDE LEVELS IMPAIR THE OLFACTORY SYSTEM OF EUROPEAN SEA BASS

■ TUESDAY 4 JULY, 2017 POSTER SESSION

● COSIMA S PORTEUS (UNIVERSITY OF EXETER, UNITED KINGDOM), PETER C HUBBARD (CENTRO DE CIÊNCIAS DO MAR, PORTUGAL), TAMSYN M UREN WEBSTER (SWANSEA UNIVERSITY, UNITED KINGDOM), RONNY VAN AERLE (CENTRE FOR ENVIRONMENT FISHERIES AND AQUACULTURE SCIENCE (CEFAS), UNITED KINGDOM), ADELINO V M CANARIO (CENTRO DE CIÊNCIAS DO MAR, PORTUGAL), EDUARDA M SANTOS (UNIVERSITY OF EXETER, UNITED KINGDOM), ROD W WILSON (UNIVERSITY OF EXETER, UNITED KINGDOM)

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The survival of marine fishes exposed to elevated near-future CO₂ levels is threatened by their altered responses to sensory cues. Here, we demonstrate a novel physiological and molecular mechanism based in the olfactory system which helps explain altered behaviour caused by elevated CO₂. Electrophysiology and high throughput sequencing were combined with behavioural experiments to investigate the effects of elevated CO₂ on the olfactory system of European sea bass (*Dicentrarchus labrax*), an economically important species. It was estimated that in elevated CO₂ (~1000 µatm) fish need to be up to 64% closer to an odour source for detection, compared with current CO₂ levels, decreasing their chances of escaping predators. These findings correlated with a suppression in the transcription of genes involved in synaptic strength, cell excitability, and learning/memory in response to elevated CO₂ exposure. Our novel findings contrast with the previously proposed brain-based mechanism and demonstrate that both olfactory and brain function are compromised by elevated CO₂ in the oceans, with potentially major negative impacts on fish globally.

A3.19 DIFFERENT LEVELS OF REDUCED OXYGEN ELICIT DIFFERENT PHYSIOLOGICAL AND TRANSCRIPTOMIC MECHANISMS IN THE BRACKISHWATER AMPHIPOD, *GAMMARUS CHEVREUXI*

■ TUESDAY 4 JULY, 2017 POSTER SESSION

● MICHAEL H COLLINS (PLYMOUTH UNIVERSITY, UNITED KINGDOM), OLIVER TILLS (PLYMOUTH UNIVERSITY, UNITED KINGDOM), JOHN I SPICER (PLYMOUTH UNIVERSITY, UNITED KINGDOM), LUCY M TURNER (PLYMOUTH UNIVERSITY, UNITED KINGDOM), MELODY S CLARK (BRITISH ANTARCTIC SURVEY, UNITED KINGDOM), MANUELA TRUEBANO (PLYMOUTH UNIVERSITY, UNITED KINGDOM)

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Whilst the physiological responses of marine invertebrates to reductions in environmental oxygen are relatively well documented, the underlying molecular mechanisms remain largely unknown, particularly in sensitive taxa like crustaceans. This study investigated the physiological and transcriptomic responses to different degrees of exposure to 7 days of hypoxia (20 and 40% air saturation) of the brackishwater amphipod *Gammarus chevreuxi*.

While amphipods were oxyregulators at both hypoxia levels, there were reductions in aerobic scope which increased with increasing severity of hypoxia. At 40% there was an increase in the expression of metabolic genes, oxygen transporters and cellular defences from which it could be inferred that this was a response to the metabolic challenge posed by low oxygen. However, there was a dramatic reversal at 20% with widespread downregulation of genes involved in metabolism and protein synthesis. This could be interpreted either as a physiologically impaired state under severe hypoxia or as an energy conservation strategy and possibly transition towards future hypometabolism.

A3.20 INDIVIDUAL REPEATABILITY OF THERMAL TOLERANCE IN ZEBRAFISH AT OPTIMAL AND WARM ACCLIMATED TEMPERATURES: A FOUNDATION FOR EVOLUTION

■ TUESDAY 4 JULY, 2017 POSTER SESSION

● RACHAEL MORGAN (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY), METTE H FINNØEN (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY), FREDRIK JUTFELT (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY)

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To address whether thermal tolerance can evolve in vertebrates, we first need to establish that there are intraspecific differences in thermal tolerance, which are consistent within an individual. Therefore, zebrafish (*Danio rerio*) were exposed to four acute thermal challenges, one week apart, and individual repeatability of thermal tolerance (critical thermal maxima, CT_{MAX}) was estimated. Additionally, the effect of warm acclimation on CT_{MAX} was investigated by exposing fish to 34°C prior to the first trial (warm acclimated) and comparing them with fish at 28°C (optimal temperature). Fish at 34°C had a higher initial CT_{MAX} than fish at 28°C (p<0.01), with CT_{MAX}'s ranging from 41.2-43.2°C compared to 39.3-41.4°C for the optimal temperature group. This indicates that short-term acclimation significantly increases thermal tolerance. Warm acclimated fish showed the highest individual repeatability between challenges (r=0.79, 95% C.I. 0.48-0.89). Repeatability was also relatively high in 28°C fish (r=0.54, 95% C.I. 0.21-0.77). As CT_{MAX} increased from the first trial to the second (optimal: +0.41°C, p=0.01; acclimated: +0.11°C p=0.02) differences in repeatability may result from 'optimal temperature' individuals responding differently to the first CT_{MAX}, whereas acclimated fish were primed for a thermal challenge. This shows that thermal tolerance consistently varies between individuals, is highly repeatable, and may provide grounds for selection.

A3.21 CHRONIC WARM EXPOSURE IMPAIRS GROWTH PERFORMANCE AND REDUCES THERMAL SAFETY MARGINS IN THE NEW ZEALAND COMMON TRIPLEFIN FISH (*FORSTERYGION LAPILLUM*)

TUESDAY 4 JULY, 2017 POSTER SESSION

TRISTAN J MCARLEY (THE UNIVERSITY OF AUCKLAND, NEW ZEALAND), ANTHONY J R HICKEY (THE UNIVERSITY OF AUCKLAND, NEW ZEALAND), NEILL A HERBERT (THE UNIVERSITY OF AUCKLAND, NEW ZEALAND)

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Fish species which occupy both intertidal and subtidal habitats face a dual challenge from climate induced warming as they will be exposed to both chronically elevated ambient temperatures and greater extremes of acute temperature change. As sea temperatures rise it has been proposed that whole animal performance will be impaired through oxygen capacity limited thermal tolerance (OCLTT, reduced aerobic metabolic scope-MS) and, on an acute scale, thermal safety margins will be reduced due to limited plasticity in upper thermal tolerance limits. Using the New Zealand common triplefin fish (*Forsterygion lapillum*), this study addressed how performance in terms of growth and metabolism (MS) and upper thermal tolerance limits would be affected by chronic exposure (12 weeks) to elevated temperature. In agreement with the OCLTT hypothesis chronic exposure to elevated temperature significantly reduced growth performance and MS. However, despite the prospect of impaired growth performance under warmer future summertime conditions an annual growth model revealed that elevated temperatures would only shift the timing of high growth potential and not the overall annual growth rate. While the upper thermal tolerance (i.e. critical thermal maxima) increased marginally with chronic exposure to warmer temperatures and was associated with depressed metabolic rates during acute thermal ramping, upper thermal tolerance did not differ between present and predicted future summertime temperatures. This suggests that warming may progressively decrease thermal safety margins for hardy generalist species and could limit the available habitat range of intertidal populations.

A3.22 DOES DEVELOPMENT AFFECT THE HEAT SHOCK RESPONSE OF THE GREEN SEA URCHIN *PSAMMECHINUS MILIARIS*?

TUESDAY 4 JULY, 2017 POSTER SESSION

ALESSANDRO CAVALLO (BRITISH ANTARCTIC SURVEY, UNITED KINGDOM), MELODY S CLARK (BRITISH ANTARCTIC SURVEY, UNITED KINGDOM), COLEEN C SUCKLING (SCHOOL OF OCEAN SCIENCES BANGOR UNIVERSITY, UNITED KINGDOM), CLARA L MACKENZIE (CENTRE FOR MARINE BIODIVERSITY AND BIOTECHNOLOGY HERIOT-WATT UNIVERSITY, UNITED KINGDOM), ELISHA SLATER (SCHOOL OF OCEAN SCIENCES BANGOR UNIVERSITY, UNITED KINGDOM), MICHAEL A S THORNE (BRITISH ANTARCTIC SURVEY, UNITED KINGDOM), ANDREW J DAVIES (SCHOOL OF OCEAN SCIENCES BANGOR UNIVERSITY, UNITED KINGDOM), LLOYD S PECK (BRITISH ANTARCTIC SURVEY, UNITED KINGDOM)

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Temperature's ubiquitous effects on biological processes encompass all levels of biological organisation. In order to make predictions on how organisms will be impacted by global warming, it is crucial to know how their molecular machinery works under different thermal regimes. Temperate marine invertebrates are useful models because they are naturally subjected to wide temperature fluctuations, and they often undergo an important ecological transition from a planktonic larval existence to a benthic one. Larval stages are often considered to be the most vulnerable, however, relatively little is known about how temperature fluctuations affect these early developmental stages. In light of this, the present study investigated the molecular response to heat shock in the green sea urchin *Psammechinus miliaris* at different times in early development. This was compared to the response in adult animals in order to identify if the developmental stage of the larvae impacted their ability to mount an effective heat shock response. The experiment exposed animals (larvae and adults) to a one-hour heat shock at 25°C. The expression of nine heat shock protein genes was used as a proxy measure for the response to cellular stress, considering the important and well-studied role of HSPs in this biological process. These results will be discussed alongside those from a recent parallel experiment on the effects of acidified sea water on larvae of the same species, allowing for an inter-stressor comparison of their molecular responses to changing environmental conditions.

A3.23 THE EPIGENETIC LANDSCAPE OF TRANSGENERATIONAL ACCLIMATION TO OCEAN WARMING

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 TIMOTHY RAVASI (KING ABDULLAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, SAUDI ARABIA), TAEWOO RYU (APEC CLIMATE CENTER, KOREA (SOUTH)), HEATHER D VEILLEUX (ARC CENTRE OF EXCELLENCE FOR CORAL REEF STUDIES JAMES COOK UNIVERSITY, AUSTRALIA), JENNIFER M DONELSON (ARC CENTRE OF EXCELLENCE FOR CORAL REEF STUDIES JAMES COOK UNIVERSITY, AUSTRALIA), PHILIP L MUNDAY (ARC CENTRE OF EXCELLENCE FOR CORAL REEF STUDIES JAMES COOK UNIVERSITY, AUSTRALIA)

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Epigenetic inheritance is a potential mechanism by which the environment experienced in one generation can influence the performance of future generations in the same environment. Rapid climate change threatens the survival of many organisms; however, recent studies show that some species can adjust to climate related stress when both parents and their offspring experience the same environmental change. In my talk, I will discuss our latest research that by means of an integrative genomics analysis, identified molecular pathways responsible for transgenerational acclimation to rising ocean temperatures of the coral reef fish *Acanthochromis polyacanthus* and shown that selective genomic-wide DNA methylation serves as a central epigenetic inheritance mechanism mediating transgenerational acclimation to climate change.

A3.24 INFLUENCE OF CHOLINERGIC INHIBITION OF HEART RATE ON THERMAL TOLERANCE IN THE ROACH, *RUTILUS RUTILUS*

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 ANDREAS EKSTRÖM (UNIVERSITY OF GOTHENBURG, SWEDEN), ERIK SANDBLOM (UNIVERSITY OF GOTHENBURG, SWEDEN)

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Declining venous (i.e., luminal) oxygen supply to the heart during warming has been proposed to result in cardiac failure and to set the critical thermal maximum (CT_{max}) in fish species that lack a coronary circulation. However, cholinergic inhibition of heart rate may enhance cardiac function at extreme high temperatures by increasing diastole and improve cardiac oxygenation by reducing myocardial diffusion distances and increase the time for oxygen diffusion. Here, we tested this hypothesis in roach (*Rutilus rutilus*), a fish species in which the heart is solely dependent on luminal oxygenation. Heart rate increased with acute warming (3°C h^{-1}) in both control fish (saline) and in roach treated with the cholinergic blocker atropine sulphate (1.2 mg kg^{-1}). While heart rate was consistently elevated across temperatures in atropinized fish, the difference in heart rate between treatment groups increased with warming indicating an increasing cholinergic tone in the untreated fish at high temperatures. However, contrary to our hypothesis, cholinergic blockade did not affect CT_{max} or the temperature where heart rate failed. This suggests that the increased cholinergic tone

with warming had no adaptive benefits for acute thermal tolerance or cardiovascular performance in roach at the quick heating rates employed here. Being a relatively hypoxia tolerant species, the roach heart may have a considerable anaerobic capacity that can buffer short-term myocardial oxygen shortage. Thus, the adaptive benefits of cholinergic inhibition of heart rate on cardiac oxygenation and thermal tolerance could be more pronounced at slower heating rates as e.g., glycogen stores are depleted.

A3.25 COMBINED EFFECTS OF OCEAN ACIDIFICATION AND TEMPERATURE ON THE SWIMMING CAPACITY OF EUROPEAN SEA BASS LARVAE

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 LOUISE COMINASSI (HAMBURG UNIVERSITY, GERMANY), MARTA MOYANO (HAMBURG UNIVERSITY, GERMANY), GUY CLAIREAUX (UNIVERSITÉ DE BRETAGNE OCCIDENTALE, FRANCE), SARAH HOWALD (ALFRED WEGENER INSTITUT, GERMANY), JOSÉ-LUIS ZAMBONINO (IFREMER, FRANCE), FELIX C MARK (ALFRED WEGENER INSTITUT, GERMANY), MYRON A PECK (HAMBURG UNIVERSITY, GERMANY)

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Swimming capacity is a key determinant of Darwinian fitness as it influences a number of fundamental attributes of performances, including the ability to acquire food, and to avoid predators. Swimming ability is also an integrated marker of the functioning of a variety of interlinked physiological functions that contribute to locomotion. For that reason, swimming capacity is generally considered a good indicator of fish health and impairments of swimming due to climate-driven changes in temperature and pH (ocean acidification) will, thus, have far-reaching consequences for fish populations. We examined the effects of ocean acidification and warming (OAW) on growth, development (e.g., morphology, ossification) and critical swimming speed (U_{crit}) of European sea bass (*Dicentrarchus labrax*) larvae. Since 2 days post hatch (dph), larvae were exposed to a combination of three levels of $p\text{CO}_2$ (400, 800, $1200\ \mu\text{atm}$; pH 8.1, 7.8, 7.6) and two temperatures (15, 20°C). Larval stage duration was 35% longer at 15°C (60 days) compared to 20°C (45 days). Thermal effects on growth and development were stronger than those of $p\text{CO}_2$ levels although the rate of ossification was higher at higher $p\text{CO}_2$. Swimming capacity increased rapidly with larval size. However, for larvae reared at 20°C , U_{crit} reached a plateau around 13 mm in standard length, resulting in lower U_{crit} at metamorphosis ($3.5\ \text{cm}\cdot\text{s}^{-1}$) compared to 15°C -reared larvae ($6.3\ \text{cm}\cdot\text{s}^{-1}$). Overall, our data suggest that swimming capacity in European seabass is impacted by temperature during larval development but not by pCO_2 .

A3.26 HEAT-INDUCED ANEMONE BLEACHING INCREASES THE OXYGEN DEMANDS OF SYMBIONT ANEMONEFISH

TUESDAY 4 JULY, 2017 POSTER SESSION

• TOMMY NORIN (UNIVERSITY OF GLASGOW, UNITED KINGDOM), SUZANNE C MILLS (CRIOBE USR 3278 EPHE-CNRS-UPVD PSL RESEARCH UNIVERSITY, FRENCH POLYNESIA), SHAUN S KILLEN (UNIVERSITY OF GLASGOW, UNITED KINGDOM), RICARDO BELDADE (CRIOBE USR 3278 EPHE-CNRS-UPVD PSL RESEARCH UNIVERSITY, FRENCH POLYNESIA)

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Increased ocean temperatures are causing mass bleaching of anemones and corals in the tropics worldwide. This heat-induced loss of algal symbionts directly affects anemones or corals physiologically, but the damage may also cascade on to other animal symbionts. We collected wild juvenile orange-fin anemonefish (*Amphiprion chrysopterus*) from around the island of Moorea, French Polynesia, and maintained the fish individually at 28°C (ambient seawater temperature) in aquaria with either bleached or unbleached magnificent sea anemones (*Heteractis magnifica*) for two weeks before measuring the oxygen uptake rates of the fish (a measure of their metabolic rates) while in the presence of their respective anemone treatments (n=10 per treatment). All of resting, night-time, and day-time oxygen uptake rates were significantly higher by 19, 19, and 23%, respectively, for fish living on bleached compared to unbleached anemones. Furthermore, total oxygen uptake during the 19 h metabolic rate trials was 31% higher for fish living on bleached anemones. These significantly higher oxygen demands suggest that the anemonefish were uncomfortable or stressed in a bleached anemone host, which may have negative effects on the fish through the increased metabolic costs incurred with potential impacts on other important life-history traits.

A3.27 THERMAL SENSITIVITY OF MITOCHONDRIAL ELECTRON TRANSPORT CHAIN ENZYMES IN WILD AND CAPTIVE BRED BROWN TROUT, *SALMO TRUTTA*

TUESDAY 4 JULY, 2017 POSTER SESSION

• BASTIEN THOMAS (UNIVERSITY OF POITIERS, FRANCE), HOLLY ALICE SHIELDS (THE UNIVERSITY OF MANCHESTER, UNITED KINGDOM), GINA L J GALLI (THE UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

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Recent evidence suggests mitochondrial dysfunction is intimately involved in setting the thermal range that ectotherms can tolerate, possibly providing a simple predictor of the likely effects of climate change. Most of these investigations have used farmed fish, whose physiology and metabolic capacity is significantly different to wild fish. To ensure reliable predictions, it is essential to collect information from wild populations. Brown trout are cold-adapted and especially vulnerable to climate change, but nothing is known about mitochondrial function in this species. To this end, we have investigated the thermal sensitivity of enzymes in the mitochondrial electron transport chain from wild and captive bred

brown trout. Tissue was harvested from both groups and frozen in liquid nitrogen for later analysis. Tissue homogenates were prepared and enzymatic activity of citrate synthase and Complexes I-V of the electron transport chain were measured at a range of temperatures with a spectrophotometer. Complex activity increased with temperature up until a threshold, at which point, enzymatic activity declined. Early results suggest the breakpoint temperature is lower in wild trout compared to captive bred. These results suggest captive bred trout are not suitable surrogates for investigating the effects of climate change on metabolic properties of wild trout.

A3.28 EFFECTS OF TEMPERATURE AND SALINITY ON THE SURVIVAL AND PHYSIOLOGY OF BALTIC *MYTILUS* SP. EARLY LIFE-STAGES

TUESDAY 4 JULY, 2017 POSTER SESSION

• JENNIFER C NASCIMENTO SCHULZE (GEOMAR HELMHOLTZ CENTRE FOR OCEAN RESEARCH KIEL, GERMANY), TRYSTAN SANDERS (GEOMAR HELMHOLTZ CENTRE FOR OCEAN RESEARCH KIEL, GERMANY), JÖRN THOMSEN (GEOMAR HELMHOLTZ CENTRE FOR OCEAN RESEARCH KIEL, GERMANY), FRANK MELZNER (GEOMAR HELMHOLTZ CENTRE FOR OCEAN RESEARCH KIEL, GERMANY)

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Low salinity conditions of brackish coastal systems represent a challenging habitat for marine organisms. In the Baltic Sea, one of the largest brackish water bodies of the world, anthropogenic induced climate change is expected to decrease salinity and elevate water temperature. Populations of *Mytilus* sp. inhabiting the southern and eastern coasts of the Baltic are found at the lower limit of their salinity tolerance range. This study aimed to understand how changes in salinity and temperature corresponding to year 2100 scenarios act on early life-stages of Baltic *Mytilus*. Mussels were collected from Ahrenshoop, Germany (salinity = 11 psu) and spawned in laboratory. Larvae were submitted to treatments combining different levels of salinity (11, 9 and 7 psu) and temperature (12 and 15°C). Rates of survival, growth and settlement were strongly reduced at salinities of 9 and 7 psu. Increased temperature (15°C) alleviated the negative effects of low salinity. Larvae could delay metamorphosis up to 67 days in adverse salinity conditions of 7 psu. Respiration rate increased with decreasing salinities of 9 and 7 psu and a synergistic effect of increased temperature was observed. Larval clearance rates decreased at low salinity treatments. These effects led to a steep reduction in scope for growth with reduced salinity. Concluding, future abiotic conditions in the Baltic Sea likely represent a bottleneck for survival and development of *Mytilus* sp. larvae, potentially redefining distribution limits of mussel populations in this area. This would have far reaching consequences for benthic ecosystems in the central Baltic Sea.

A3.29 OXYGEN TRANSPORT SYSTEM FROM MOLECULAR TO FUNCTIONAL LEVEL; DIFFERENCES BETWEEN THERMALLY HIGH AND LOW TOLERANT EUROPEAN SEABASS

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 KATJA ANTTILA (UNIVERSITY OF TURKU, FINLAND), FLORIAN MAUDUIT (CENTRE IFREMER DE BRETAGNE, FRANCE), MIRELLA KANERVA (UNIVERSITY OF TURKU, FINLAND), GUY CLAIREAUX (UNIVERSITÉ DE BRETAGNE OCCIDENTALE, FRANCE), MIKKO NIKINMAA (UNIVERSITY OF TURKU, FINLAND)

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One of the key features of fish responding to environmental changes is adjusting respiratory and circulatory system. Therefore, complete understanding of the function of oxygen transport system is necessary for predicting, e.g., how fish can deal with climate change. To advance this understanding, we compared seabass that had significantly different critical thermal maxima (CT_{max} ; measured by heating the water from 15 to 30°C in 2h and from 30°C to 34°C in 4h). 621 European seabass were tested for CT_{max} and the extreme individuals (time for losing equilibrium 2.5 ± 0.03 h [untolerant] vs. 5.0 ± 0.03 h [highly tolerant]) were selected for further testing after a three-week recovery. Fish were subjected to heat shock (warming 15 → 28°C within 1.5h) and sampled before and after the shock. Highly tolerant individuals had larger ventricles, higher maximum heart rate, higher ventricular Ca^{2+} -ATPase and lactate dehydrogenase activities as compared to intolerant individuals indicating higher blood pumping capacity. The lengths of gill secondary lamellae and capillary densities of muscle were similar in the groups. However, the intolerant individuals had bigger myocytes with lower myoglobin level than the highly tolerant ones indicating limited oxygen extraction capacity in muscular level. The intolerant individuals also had higher liver oxidative stress enzyme activities (glutathione peroxidase and reductase and glucose-6-phosphate-dehydrogenase) suggesting a more serious disturbance of redox balance by heat stress than in the highly tolerant specimens. As conclusion, the molecular differences in cardiomyocyte level translate to functional differences which may have a role in determining the upper thermal tolerance of fish.

A3.30 CHANGES IN METABOLOME AND MITOCHONDRIAL RESPIRATION IN EUROPEAN SEA BASS HEARTS UNDER OAW

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 SARAH HOWALD (ALFRED-WEGENER-INSTITUTE BREMERHAVEN, GERMANY), SARAH KEMPF (ALFRED-WEGENER-INSTITUTE BREMERHAVEN, GERMANY), HANNA SCHEUFFELE (ALFRED-WEGENER-INSTITUTE BREMERHAVEN, GERMANY), GUY CLAIREAUX (UNIVERSITÉ DE BRETAGNE OCCIDENTALE, FRANCE), HÉLÈNE OLLIVIER (UNIVERSITÉ DE BRETAGNE OCCIDENTALE, FRANCE), LOUISE COMINASSI (UNIVERSITY OF HAMBURG, GERMANY), NICOLAS LE BAYON (IFREMER, FRANCE), CHRISTIAN BOCK (ALFRED-WEGENER-INSTITUTE BREMERHAVEN, GERMANY), FELIX C. MARK (ALFRED-WEGENER-INSTITUTE BREMERHAVEN, GERMANY)

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Rising temperatures and atmospheric pCO_2 lead to a warmer and more acidic ocean and therefore to the need to predict how marine ectotherm species will cope with future ocean conditions. The European sea bass *Dicentrarchus labrax* is an economically important species inhabiting temperate coastal waters of the Mediterranean and North Atlantic. We raised sea bass larvae until juvenile stage in two temperature conditions (ambient and ambient plus 5°C) combined with three acidification conditions (400, 800 and 1200 μ atm CO_2). The metabolic fingerprints of the juvenile fish showed decreases in amino acid and osmolyte concentrations with increasing temperature, except for an increase in Leucine. Energy reserve metabolites, as well as stress indicators were not altered by changing temperature, while hypercapnia led to a decrease in ADP and ATP. Organic acids were not altered by experimental conditions. The changes in amino acid concentration under hypercapnia and increased temperature might indicate an increase in protein catabolism. However, no signs of increased anaerobic metabolism or decreased energy reserves were observed. While the metabolome was altered due to acidification, the respiratory capacity of heart mitochondria was not changed due to acidification. However, increasing acclimation temperature led to decreasing mitochondrial efficiency, reflected by decreases in OXPHOS capacity, complex IV respiration and total ETS (electron transport system) respiration. Nevertheless, proton leak was even more decreased with increasing temperature, which led to an increase in respiratory control ratio (RCR+). In conclusion the European sea bass might cope well with increasing ocean acidification, while rising temperatures could lower its fitness.

A3.31 IMPACT OF OCEAN ACIDIFICATION ON THE EARLY DEVELOPMENT AND SHELL MINERALIZATION OF THE EUROPEAN ABALONE (*HALIOTIS TUBERCULATA*)

TUESDAY 4 JULY, 2017 POSTER SESSION

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Ocean acidification is a major global stressor that leads to substantial changes in seawater carbonate chemistry, with potential significant consequences for calcifying organisms. Marine shelled molluscs are ecologically and economically important species providing essential ecosystem services and food sources. Because they use calcium carbonate (CaCO_3) to produce shells, molluscs are among the most vulnerable invertebrates to ocean acidification, with early developmental stages being particularly sensitive to pH changes. Early development and shell formation have been extensively studied in *H. tuberculata*, showing that the primary shell is mostly composed of amorphous CaCO_3 , followed by a gradually crystallization under aragonite. Since aragonite is more susceptible to dissolution compared to calcite, the abalone shell provides a relevant model to study the impact of ocean acidification.

This study investigated the effects of CO_2 -induced ocean acidification on the European abalone *Haliotis tuberculata*, a commercially important gastropod species. Larval and juvenile abalones, obtained from controlled fertilization held at the hatchery France-Haliotis, were submitted to a range of decreased pHs (8.1 to 7.6) over the development cycle. Biological responses were evaluated by measuring survival, development, growth index and shell calcification. Polarized and SEM microscopy were used to assess whether lowering the pH had an influence on shell morphology and microstructure. Our results evidenced that ocean acidification negatively impacted abalone development and disrupted the shell formation process.

Since these biological effects were observed for pH values expected by 2100, ocean acidification may have potential negative consequences for abalone recruitment and persistence of wild populations in a near future.

A3.32 INTERTIDAL OYSTERS REACH THEIR PHYSIOLOGICAL LIMIT IN A FUTURE HIGH CO_2 WORLD

TUESDAY 4 JULY, 2017 POSTER SESSION

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Sessile marine molluscs living in the intertidal zone experience periods of internal acidosis when exposed to air (emersion) during low tide. Relative to other marine organisms, molluscs have been identified as vulnerable to future ocean acidification, however, paradoxically it has also been shown that molluscs exposed to high CO_2 environments are more resilient compared to those molluscs naïve of CO_2 exposure. Two competing hypotheses were tested using a novel experimental design incorporating tidal simulations to predict the future intertidal limit of oysters in a high CO_2 world; either high-shore oysters will be more tolerant of elevated $p\text{CO}_2$ because of their regular acidosis, or elevated $p\text{CO}_2$ will cause high-shore oysters to reach their limit. Sydney rock oysters, *Saccostrea glomerata*, were collected from the high-intertidal and subtidal areas of the shore and exposed in an orthogonal design, to either an intertidal or subtidal treatment at ambient or elevated $p\text{CO}_2$, and physiological variables were measured. The combined treatment of tidal emersion and elevated $p\text{CO}_2$ interacted synergistically to reduce the pHe of oysters, increase PeCO_2 , and standard metabolic rate. Oysters in an intertidal treatment also had lower condition and growth. Oysters showed a high degree of plasticity, and little evidence was found that intertidal oysters were more resilient than subtidal oysters. It is concluded that in a high CO_2 world the upper vertical limit of oyster distribution on the shore may be reduced. These results suggest that previous studies on intertidal organisms which lack tidal simulations, may have underestimated the effects of elevated $p\text{CO}_2$.

A3.33 LESSONS FROM TWO HIGH CO₂ WORLDS: FUTURE OCEANS AND INTENSIVE AQUACULTURE

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 ROD W WILSON (UNIVERSITY OF EXETER, UNITED KINGDOM), ROBERT P ELLIS (UNIVERSITY OF EXETER, UNITED KINGDOM), MAURICIO A URBINA (UNIVERSIDAD DE CONCEPCIÓN, CHILE)

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Exponentially rising CO₂ is a global concern, driving climate change and causing acidification of both marine and freshwater environments. Physiologists have long known CO₂ directly affects acid-base and ion regulation, respiratory and metabolic function. More recently, many studies have demonstrated that elevated CO₂ projected for end of this century (e.g. 800–1,000 µatm) has additional, previously unforeseen, effects on sensory and nervous system functions of fish and invertebrates, negatively impacting behaviour, fitness, and survival.

Despite this, elevated CO₂ is also intimately associated with intensive aquaculture. Production demand and stocking density can elevate CO₂ levels beyond 10,000 µatm in many production systems. Understanding potential physiological implications of these extreme CO₂ conditions is thus crucial for the optimisation of intensive aquaculture practices, and ultimately for ensuring this sector is able to sustainably intensify production to meet the increasing global demand.

Whilst high CO₂ is of relevance to both intensive aquaculture and aquatic acidification, traditionally these two connected fields have remained disparate. We highlight the importance of bringing these communities together, and of delineating a pathway for positive interaction that can direct future research for mutual benefit. This in turn will improve understanding of the negative impacts of CO₂, as well as enable the optimisation of aquaculture practices and animal welfare. The future challenge of managing disease in global aquaculture and the development of successful mitigation strategies are crucial to support an expanding and sustainable industry towards 2050, understanding of the impact of water quality on organism physiology is imperative to enable this process.

A3.34 NO EVIDENCE THAT ELEVATED CO₂ AFFECTS BEHAVIOURAL LATERALIZATION, ACTIVITY, AGGRESSION OR MONOAMINE NEUROTRANSMITTER LEVELS IN THE THREE-SPINED STICKLEBACK (GASTEROSTEUS ACULEATUS)

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 LAURA E VOSSEN (UPPSALA UNIVERSITY, SWEDEN), FREDRIK JUTFELT (NORWEGIAN UNIVERSITY FOR SCIENCE AND TECHNOLOGY, NORWAY), JOHAN RUDIN (UPPSALA UNIVERSITY, SWEDEN), JOSEFIN SUNDIN (UPPSALA UNIVERSITY, SWEDEN), SVANTE WINBERG (UPPSALA UNIVERSITY, SWEDEN)

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Exposure to near-future CO₂ concentrations has been reported to cause behavioural disruptions in marine teleost fish, including hyperactivity, changes in behavioural lateralization and perhaps most strikingly, attraction to predator odour. These changes have been hypothesized to result from a reversal in the function of the main inhibitory neurotransmitter in vertebrates, GABA. Treatment with the GABA A receptor antagonist gabazine has been shown to restore the behaviour of CO₂ affected fish. However, in these studies gabazine did not affect behaviour under control CO₂. We investigated whether the gabazine concentration used in most studies (4 mg/L for 30 minutes) affected locomotory activity, in comparison to both a lower (0.8 mg/L) and a higher (40 mg/L) dose. We also tested activity, anxiety and lateralization after exposure to control (450 µatm) or elevated (1000 µatm) pCO₂, using high sample sizes and automated tracking software. Finally, we quantified territorial aggression, not previously investigated in CO₂ exposure studies, and analysed monoamine neurotransmitter levels that are involved in aggressive behaviour and are under the control of GABA. Our results suggest that the most commonly used gabazine dose is too low to affect behaviour in the three-spined stickleback, whereas a ten times higher dose induces hyperactivity. None of the behaviours investigated, or the monoamine neurotransmitter levels, differed significantly between CO₂ exposure groups.

A3.35 TOWARDS UNDERSTANDING SUB LETHAL EFFECTS OF CLIMATE CHANGE ON MARINE CRUSTACEANS

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 HANNAH WOOD (UNIVERSITY OF GOTHENBURG, SWEDEN), SUSANNE P ERIKSSON (UNIVERSITY OF GOTHENBURG, SWEDEN)

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The interaction of climate related environmental stressors and the subsequent effects on marine species presents a major threat to future biodiversity within our oceans. Here we present results on how a variety of climate stressors, alone and in combination, impact upon the health and fitness of marine crustacean. This work encompasses comparative studies at the individual and population level, and climate stressors including ocean acidification, ocean warming and hypoxia.

A3.36 THERMAL SENSITIVITY AT CONSTANT TEMPERATURES DOES NOT PREDICT RESPONSES UNDER VARYING TEMPERATURES

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 KATIE E MARSHALL (UNIVERSITY OF OKLAHOMA, UNITED STATES), KATHRYN M ANDERSON (UNIVERSITY OF BRITISH COLUMBIA, CANADA), JOEY R BERNHARDT (UNIVERSITY OF BRITISH COLUMBIA, CANADA), NORAH E BROWN (UNIVERSITY OF BRITISH COLUMBIA, CANADA), JACOB K DYTNERSKI (HONG KONG UNIVERSITY, CHINA), KELSEY L FLYNN (UNIVERSITY OF BRITISH COLUMBIA, CANADA), HELEN GURNEY-SMITH (VANCOUVER ISLAND UNIVERSITY, CANADA), CASSANDRA A KONECNY (UNIVERSITY OF BRITISH COLUMBIA, CANADA), CHRISTOPHER DG HARLEY (UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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Predicting the effects of climate change on organisms' physiology, fitness, and potential geographical distribution relies on a clear understanding of the effects of temperature on fitness measures. While organisms live in environments that regularly fluctuate in temperature, most studies on temperature sensitivity have focused on the effects of constant temperature conditions, which may have very different physiological effects. In this study we compared the responses of the bay mussel *Mytilus trossulus* from Tofino, British Columbia (outer coast) and Port Moody, British Columbia (inner coast) to either six weeks of constant temperature acclimation at 6, 12, or 18°C or to temperatures that regularly fluctuated between 6 and 18°C (with a mean of 12°C). In a diverse suite of fitness measures (feeding and heart rate, growth rate, survival, and byssal thread production), we found that responses to variable temperatures were always significantly different than to constant temperature acclimation. In particular, growth rates and survival under fluctuating conditions were significantly elevated compared to all constant conditions. In addition, each population showed unique responses to thermal acclimation, with mussels from Tofino being generally less sensitive to temperature than the mussels from Port Moody. These results suggest that projections of species responses to climate change based on experiments involving constant temperatures are likely to inadequately capture the complexity of responses under more natural, fluctuating conditions.

A3.37 FROM THE MITOCHONDRIA TO THE INDIVIDUAL: HOW TEMPERATURE INFLUENCES PERFORMANCES IN JUVENILE SEA BASS

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 CHRISTEL LEFRANÇOIS (UNIVERSITY OF LA ROCHELLE-LIENSS UMR 7266, FRANCE), LOIC TEULIER (UNIVERSITY OF LYON 1- LEHNA UMR 5023, FRANCE), MARIE VAGNER (CNRS LA ROCHELLE-LIENSS UMR 7266, FRANCE), DAMIEN ROUSSEL (UNIVERSITY OF LYON 1- LEHNA UMR 5023, FRANCE), QUENTIN TERNON (UNIVERSITY OF LA ROCHELLE-LIENSS UMR 7266, FRANCE), CAROLINE ROMESTAING (UNIVERSITY OF LYON 1- LEHNA UMR 5023, FRANCE), EMANUEL DUBILLOT (UNIVERSITY OF LA ROCHELLE-LIENSS UMR 7266, FRANCE), YAN VOITURON (UNIVERSITY OF LYON 1- LEHNA UMR 5023, FRANCE)

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The aim of our study was to investigate the links between individual performance (in terms of swimming and aerobic scope) and mitochondrial bioenergetics in fish, since mitochondria are essential organelles responsible for the generation of chemical energy in the form of ATP. We explored these links in juvenile sea bass acclimated to three different temperatures. The temperatures were chosen in regards to the temperature considered as optimal in terms of aerobic scope for this species: 22°C as the optimal level, 18 and 26°C as sub- and sub-optimal conditions, respectively. Oxygen consumption was measured in resting and active individuals during a step-protocol swimming test. Concomitantly, swimming performance was assessed in each individual through U_{max} , the swimming speed from which the fish presents burst and glide swimming mode associated to recruitment of white anaerobic muscles, as well as U_{crit} , the swimming speed at which the fish get exhausted. Afterwards, red muscles were collected in each individual and bioenergetics parameters were investigated using high resolution respirometers. Oxidative phosphorylation activity at different states, ATP synthesis and mitochondrial efficiency were measured by this way in both muscle fibers and isolated mitochondria. The bioenergetics signature at these sub-individual levels and fish performances were analyzed in regards of the temperature. In addition, inter-individual variability in performance will be discussed in relation to the pattern observed at the muscle and the mitochondrial levels.

A3.38 LARVAE VS JUVENILES: UNDERSTANDING IMPLICATIONS OF GLOBAL CHANGE THROUGHOUT THE EARLY LIFE STAGES OF THE AMERICAN LOBSTER *HOMARUS AMERICANUS*

TUESDAY 4 JULY, 2017 POSTER SESSION

FANNY NOISETTE (UNIVERSITÉ DU QUÉBEC Á RIMOUSKI, CANADA), MATHILDE CHEMEL (UNIVERSITÉ DU QUÉBEC Á RIMOUSKI, CANADA), SARAH PIEDALUE (UNIVERSITÉ DU QUÉBEC Á RIMOUSKI, CANADA), KAYLA MENU-COUREY (UNIVERSITÉ DU QUÉBEC Á RIMOUSKI, CANADA), DOUNIA DAUD (HOMARUS INC, CANADA), TAMMY BLAIR (SAINT ANDREWS BIOLOGICAL STATION DFO, CANADA), KUMIKO AZETSU-SCOTT (DALHOUSIE UNIVERSITY, BEDFORD INSTITUTE FOR OCEANOGRAPHY DFO, CANADA), PIERO CALOSI (UNIVERSITÉ DU QUÉBEC Á RIMOUSKI, CANADA)

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Marine species with complex life cycle are considered among the most sensitive to the global change because they face various environmental challenges by occupying different habitats and go through complex metamorphoses. Among the different global change drivers, the ongoing decrease in ocean pH (i.e. ocean acidification (OA)) represents a potential threat to marine ectotherms. Living and developing under low pH/ elevated pCO₂ conditions can exert negative impacts on fundamental functions such as respiration, cellular maintenance and repair, development and calcification. Thus, investigating the physiological responses of developing marine calcifying invertebrates with complex life cycles is particularly important to define marine biodiversity responses to global changes. Among such species, the American lobster is one of the most economically important for the Canadian fishing industry. We characterized life history and physiological responses of lobster larvae and juveniles (five stages) exposed throughout their entire development to one of seven seawater pH/pCO₂ conditions: mimicking current, end-of-the-century, and extreme events/ industrial leakages scenarios. Our results show that decrease in pH globally decreases the survival and carapace characteristics at all stages. Interestingly, the metabolism of stage III larvae increases with the decrease in pH, maybe due to the challenge to undergo organogenesis requirements before the metamorphosis under stressful conditions. Altogether, our results suggest that pelagic larvae are tolerant to neither OA nor extreme events while life history traits of juveniles are more negatively impacted. This suggests that carry-over or cumulative effects might affect the stages close to metamorphosis, ultimately impacting recruitment level and the lobster industry.

A3.39 HYPOXIA TOLERANCE UNAFFECTED BY INCREASED ENVIRONMENTAL CO₂ IN ACTIVE SQUIDS

TUESDAY 4 JULY, 2017 POSTER SESSION

MATTHEW A BIRK (UNIVERSITY OF SOUTH FLORIDA, UNITED STATES), ERIN L MCLEAN (UNIVERSITY OF RHODE ISLAND, UNITED STATES), BRAD A SEIBEL (UNIVERSITY OF SOUTH FLORIDA, UNITED STATES)

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Anthropogenic CO₂ emissions are increasing both ocean surface temperature and PCO₂. There has been concern that these environmental changes will impair the metabolic physiology of squids due primarily to their high metabolic needs and highly pH-sensitive blood pigments. Environmental hypoxia strains oxygen acquisition and thus hypoxia tolerance should serve as a sensitive indicator of the impact of temperature and CO₂ on oxygen supply and metabolism. In this study, aquatic respirometry was utilized to examine the effect of experimental CO₂ conditions and temperature on the respiration, metabolic rate, and hypoxia tolerance of two active squid species: the jumbo squid, *Dosidicus gigas*, and the longfin inshore squid, *Doryteuthis pealeii*. We found that increased temperature within the natural surface temperature range of *Dosidicus gigas* impaired their hypoxia tolerance and may help explain their distribution patterns. However, CO₂ had no effect on metabolic rate or hypoxia tolerance in either species. Furthermore, we consider oxygen transport parameters (e.g. Bohr coefficient, blood P50) from the literature and quantify an estimate of a "worst-case scenario" for the effect of anthropogenic CO₂ emissions on squid hypoxia tolerance. This scenario demonstrates that the ineffectiveness of CO₂ found in this study is expected. Finally, interspecific mechanistic and functional differences in the oxygen transport systems of the two species are examined to highlight the effect of the environment on physiological thresholds and limitations.

A3.40 EFFECT OF HYPOXIA FOLLOWING EXPOSURE TO HYDROCARBONS ON THE ESCAPE PERFORMANCE AND POLYCYCLIC AROMATIC HYDROCARBONS BIOCONCENTRATIONS IN A TELEOST FISH

TUESDAY 4 JULY, 2017 POSTER SESSION

THOMAS MILINKOVITCH (ISTITUTO PER L'AMBIENTE MARINO COSTIERO DEL CONSIGLIO NAZIONALE DELLE RICERCHE (IAMC-CNR) ORISTANO, ITALY), STÉPHANE LE FLOCH (CENTRE DE DOCUMENTATION DE RECHERCHE ET D'EXPÉRIMENTATION SUR LES POLLUTIONS ACCIDENTELLES DES EAUX, FRANCE), STEFANO MARRAS (ISTITUTO PER L'AMBIENTE MARINO COSTIERO DEL CONSIGLIO NAZIONALE DELLE RICERCHE (IAMC-CNR) ORISTANO, ITALY), CAMILLE LACROIX (CENTRE DE DOCUMENTATION DE RECHERCHE ET D'EXPÉRIMENTATION SUR LES POLLUTIONS ACCIDENTELLES DES EAUX, FRANCE), MORGANE DANION (AGENCE NATIONALE DE SÉCURITÉ SANITAIRE DE L'ALIMENTATION DE L'ENVIRONNEMENT ET DU TRAVAIL, FRANCE), FABIO ANTOGNARELLI (ISTITUTO PER L'AMBIENTE MARINO COSTIERO DEL CONSIGLIO NAZIONALE DELLE RICERCHE (IAMC-CNR) ORISTANO, ITALY), PAOLO DOMENICI (ISTITUTO PER L'AMBIENTE MARINO COSTIERO DEL CONSIGLIO NAZIONALE DELLE RICERCHE (IAMC-CNR) ORISTANO, ITALY)

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Among anthropogenic pressures of coastal marine ecosystems, two major growing threats are represented by hypoxia and oil pollution. These two major threats are particularly presents and coexist in marine coastal ecosystems. However, very little is known about their interactive effects on fish and other marine organisms. However, in the context of climate change, more research is needed on the impact of multiple drivers. This work provides new insights into the effects of hypoxia on the toxicological mechanisms of hydrocarbon decontamination and an evaluation of the impact of these stressors on locomotor and behavioral performances within the context of predator-prey interactions. The experiments were conducted on a teleost fish species of ecological and economic importance, the seabass *Dicentrarchus labrax*. Polycyclic aromatic hydrocarbons (PAHs) concentrations in fish liver were measured, and compared to biliary metabolites levels after decontamination in hypoxia. Our results provide information about the potential resilience of hypoxic ecosystems following oil contamination. Furthermore, we investigated the effects of hypoxia on contaminated individuals by measuring their ability to escape predators. Escape response performance was assessed through several variables: responsiveness (i.e. the proportion of animals that responded to the startling stimulation); response latency; directionality (i.e. 'away' or 'towards' the stimulus); propulsive performance (i.e. distance covered, speed and acceleration); maneuverability (i.e. turning radius, turning rate and turning angle). Our results will be discussed within the context of the ecological significance of the effects of hypoxia and hydrocarbon contamination on fish functional integrity and on their vulnerability to predation.

A4 CHALLENGES IN THE ANTHROPOCENE: ACID-BASE/ION REGULATION AND CALCIFICATION IN AQUATIC INVERTEBRATES

ORGANISED BY: DIRK WEIHRAUCH (UNIVERSITY OF MANITOBA, CANADA), MARIAN HU (KIEL UNIVERSITY, GERMANY), KATI MICHALEK (SAMS, UK) AND JAMES MORRIS (ROYAL BELGIAN INSTITUTE OF NATURAL SCIENCES, BELGIUM)

A4.1 DIGESTION AT PH 10: ECO-DEVO OF ALKALINE DIGESTIVE SYSTEMS IN MARINE LARVAE

📅 THURSDAY 6 JULY, 2017 ⌚ 09:00

👤 MEIKE STUMPP (CHRISTIAN-ALBRECHTS UNIVERSITY KIEL, GERMANY)

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Digestion is a fundamental process common to all metazoans. Most Echinoderm larvae are planktotrophic and have a simple tripartite digestive system. Depending on the species, Echinoderm and Hemichordate larvae generate highly alkaline (pH 8.5-10.4) conditions through ATP-consuming ion regulatory processes (Na⁺ K⁺-ATPase) in their digestive systems. The energetically costly regulation of gastric pH is beneficial for the breakdown of algal proteins by proteases and other digestive enzymes and is suggested to be critically involved in larval immunity. It has, therefore, a double sided role as an energy source (provision of nutrients/protection from pathogens) and energy sink (maintenance of alkaline pH) in larval life. The functionality of the larval gut is highly regulated under environmental fluctuations (e.g. pH and food), and, consequently, the alkaline gastric pH can be regarded as a key trait with outstanding significance for animal-environment interactions. Using marine larvae we developed highly tractable models to study the link between energetics and key traits that set the tolerance limits to environmental change. This information will help us to understand and predict patterns of sensitivity/resilience of echinoderm larvae in future marine habitats.

A4.2 THE IMMINENT THREAT OF FRESHWATER ACIDIFICATION TO JUVENILE LIFE STAGES OF CRUSTACEANS

📅 THURSDAY 6 JULY, 2017 ⌚ 09:40

👤 ALEX R QUIJADA-RODRIGUEZ (UNIVERSITY OF MANITOBA, CANADA), YUNG-CHE TSENG (ACADEMIA SINICA, TAIWAN), POU-LONG KUAN (ACADEMIA SINICA, TAIWAN), PO-HSIUAN SUNG (ACADEMIA SINICA, TAIWAN), MAO-TING HSU (ACADEMIA SINICA, TAIWAN), PUNG PUNG HWANG (ACADEMIA SINICA, TAIWAN), DIRK WEIHRAUCH (UNIVERSITY OF MANITOBA, CANADA)

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Climate change is occurring at an unprecedented rate and predicted to drive changes in pCO₂ of both marine and freshwater systems leading to aquatic acidification. Based on ocean acidification studies, it is believed calcifying organism will be susceptible to future CO₂ mediated acidification. However, the impacts of CO₂ mediated freshwater acidification on freshwater calcifying organisms have gone completely unstudied. In the present study, we investigated the acclimation abilities of the Chinese mitten crab *Eriocheir sinensis* to CO₂ mediated freshwater acidification through elevation of pCO₂ levels from pCO₂ levels of 100-390 Pa currently experienced in their native habitat to a potential future level of 530 Pa. We present data on hemolymph acid-base status demonstrating over a week of high pCO₂ treatment, pHe initially declines with a concomitant increase in hemolymph pCO₂. However, at the end of the week exposure the crabs appear to fully compensate for acid distress partially through accumulation of hemolymph bicarbonate and excretion of acid equivalents through the anterior gills. Coupled with compensation of pHe, we observed increases in hemolymph ammonia and ammonia excretion rates. Additionally, from a metabolic and behaviour standpoint reductions in MO₂ were observed and coupled with reduced locomotory activity. Through measurement of carapace calcium content we also demonstrate long-term freshwater acidification leads to carapace decalcification in this species. The results of this study suggest that while acid-base status can be compensated it occurs at a cost as behaviour, metabolism and calcification appear negatively impacted by freshwater acidification and may lead to detrimental effects in this species.

A4.3 COMPARATIVE STUDIES OF AMMONIA REGULATION IN GILLS OF CEPHALOPODS

THURSDAY 6 JULY, 2017 09:55

YUNG-CHE TSENG (MARINE RESEARCH STATION INSTITUTE OF CELLULAR AND ORGANISMIC BIOLOGY ACADEMIA SINICA, TAIWAN), MARIAN Y HU (INSTITUTE OF PHYSIOLOGY, UNIVERSITY OF KIEL, GERMANY), PO-HSIUAN SUNG (MARINE RESEARCH STATION INSTITUTE OF CELLULAR AND ORGANISMIC BIOLOGY ACADEMIA SINICA, TAIWAN), MENG-WEI LIN (INSTITUTE OF LIFE SCIENCE NATIONAL TAIWAN UNIVERSITY, TAIWAN), DIRK WEIHRAUCH (DEPARTMENT OF BIOLOGICAL SCIENCES, UNIVERSITY OF MANITOBA, CANADA)

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In contrast to terrestrial animals most aquatic species can tolerate relatively higher blood NH_4^+ concentrations despite its potential toxicity. Although many aquatic species can excrete NH_4^+ via specialized epithelia little information is available regarding the mechanistic basis for NH_4^+ homeostasis in molluscs.

Cephalopods have successfully evolved different lifestyles to accommodate their own ecological niches in benthic and pelagic habitats. The cephalopod gill has proved to represent the major excretory organ thus we hypothesize that cephalopods evolved diverse mechanisms for ammonia regulation for their different lifestyles. We developed an ex-vivo system to study branchial NH_4^+ transport in isolated gills of octopus, cuttlefish and squid. The gills in three species possess a bi-phasic NH_4^+ regulation. In octopus and cuttlefish gills, NH_4^+ is excreted at blood NH_4^+ levels higher than $300\ \mu\text{M}$ and increased via ammoniogenesis at NH_4^+ levels lower than $300\ \mu\text{M}$. In contrast, squid gills excreted NH_4^+ at blood NH_4^+ level higher than $100\ \mu\text{M}$ and accumulated NH_4^+ at blood NH_4^+ levels lower than $100\ \mu\text{M}$. Further experiments observed that the machinery of H^+ secretion coupled with NH_4^+ excretion can be only observed in octopus gills. Moreover, the rates of NH_4^+ excretion were higher in squid gills compared to the other two species. The NH_4^+ levels were higher ($\sim 300\ \mu\text{M}$) in octopus and cuttlefish blood compared to those of squid ($\sim 25\ \mu\text{M}$). These results inferred that the variations in ammonia homeostasis in these three cephalopods are probably linked to their respective locomotory capacities.

A4.4 VENTING OFF STRESS: WHOLE ANIMAL AND BRANCHIAL ACID-BASE REGULATORY CAPACITY OF THE SHALLOW HYDROTHERMAL VENT CRAB, XENOGRAPSUS TESTUDINATUS

THURSDAY 6 JULY, 2017 10:10

GARETT JP ALLEN (UNIVERSITY OF MANITOBA, CANADA), POU-LONG KUAN (ACADEMIA SINICA, TAIWAN), PEI-HSIUAN CHOU (ACADEMIA SINICA, TAIWAN), YUNG-CHE TSENG (ACADEMIA SINICA, TAIWAN), PUNG-PUNG HWANG (ACADEMIA SINICA, TAIWAN), DIRK WEIHRAUCH (UNIVERSITY OF MANITOBA, CANADA)

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Kueishan Island, Taiwan is home to one of the most acidic shallow hydrothermal vent systems in the known ocean where certain vent emissions reach pH 1.52, due to a mixture of volcanic gases, and 116°C . Areas surrounding vents have been documented within pH 5.5–6.5 where temperatures exceed 30°C and are home to the shallow hydrothermal vent crab, *Xenograpsus testudinatus*. *X. testudinatus* is known to migrate around vent zones, abnormally leaving vent-areas, whilst regularly feeding in close proximity to vent-openings and thus must be capable of rapid acid-base regulation. Fourteen day acclimation of *X. testudinatus* to CO_2 -induced acidification of seawater (30°C) to pH 6.50 (control; $2.7\ \text{kPa pCO}_2$) and 5.50 (treatment; $24.6\ \text{kPa pCO}_2$) was performed to determine the organisms capacity of acid-base regulation in regards to CO_2 , HCO_3^- , and H^+ -equivalents. Control crab hemolymph was stable at pH 7.47 and maintained approximately $20\ \text{mmol l}^{-1} [\text{HCO}_3^-]$, $2.1\ \text{kPa pCO}_2$, as well as interestingly high ammonia ($1.56\ \text{mmol l}^{-1}$). Acute high pCO_2 exposure caused significant acidification of hemolymph ($\Delta\text{pH} = -0.2$) accompanied by rapid accumulation of hemolymph HCO_3^- ($67\ \text{mmol l}^{-1}$; 24 hours). Within seven days of exposure *X. testudinatus* fully compensated hemolymph pH where circulating HCO_3^- stabilized at $90\ \text{mmol l}^{-1}$ ($9.6\ \text{kPa pCO}_2$) and ammonia concentration decreased ($1.26\ \text{mmol l}^{-1}$). Perfused gills of control-acclimated crabs indicated a single-pass alkalization of perfusate ($\Delta\text{pH} = +0.35$) and net HCO_3^- loss. Alkalization was negligible upon acute exposure of gills to pH 5.50 ($\Delta\text{pH} = +0.04$) linked to an increased loss of HCO_3^- .

A4.5 IT'S ALL ABOUT BALANCE: ACID-BASE REGULATION IN MARINE CRABS

📅 THURSDAY 6 JULY, 2017 ⌚ 10:55

👤 SANDRA FEHSENFELD (UNIVERSITY OF BRITISH COLUMBIA, CANADA), STEPHANIE HANS (UNIVERSITY OF MANITOBA, CANADA), DIRK WEIHRAUCH (UNIVERSITY OF MANITOBA, CANADA)

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As one of the largest invertebrate groups with an estimated more than 14,000 species, aquatic decapod crustaceans can be found virtually in all water bodies around the world. Often encountering a highly variable and constantly changing environment (i.e. estuaries), effective (physiological) mechanisms need to be in place allowing these animals to acclimate to, and counteract, physiological disturbances. One of the key processes ensuring performance and homeostasis with this respect is acid-base regulation. Like other aquatic animals (i.e. fish, cephalopods), many decapod crustaceans can counteract disturbances of acid-base homeostasis by adjusting hemolymph bicarbonate levels and the excretion of acid and/or base equivalents to secure stable extra- and intracellular pH. Being the major site of ion transport processes in crustaceans, an important component of these regulatory mechanisms is the gill epithelium as the boundary layer - and hence "translator" - between the outside and inside of the animal. Exhibiting the full range of physiological strategies from being osmo-conformers to being highly efficient hypo-hyper-regulators, however, raises the question of how these mechanisms might vary with life strategy. In this talk, I will present our current knowledge and understanding of acid-base regulatory mechanisms in decapod crustaceans. Furthermore, I will demonstrate the close link between acid-base and ammonia regulation by discussing important epithelial transporters, including the newly identified hyperpolarization-activated cyclic nucleotide-gated potassium channel 2 (HCN2).

A4.6 HAEMOLYMPH PCO₂ AND TCO₂ IN THE AQUATIC AND TERRESTRIAL LIFE STAGES OF AESHNID DRAGONFLIES

📅 THURSDAY 6 JULY, 2017 ⌚ 10:55

👤 PHILIP G D MATTHEWS (UNIVERSITY OF BRITISH COLUMBIA, CANADA), DANIEL J LEE (UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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Dragonflies begin their lives as aquatic nymphs that breathe water using a rectal gill before emerging as an air-breathing imago (flying adult) during their final molt. While the respiratory consequences of a water-to-air transition have been well studied in vertebrates and crustaceans, the respiratory changes that developmentally amphibious insects undergo are virtually unknown. In this study, total CO₂ (TCO₂) levels were measured using samples of haemolymph extracted from water-breathing aeshnid dragonfly nymphs and air-breathing adults, while fiber optic CO₂ sensors implanted directly into the haemocoel were used to monitor nymph haemolymph PCO₂ in vivo over 24h. These measurements were also repeated on a fully aquatic arthropod: the marbled crayfish (*Procambarus fallax*). Dragonfly nymphs had a very high haemolymph TCO₂ (14-21 mmol/L, depending on developmental stage) compared with other water-breathing arthropods, while the water-breathing final instar had a TCO₂ that was not significantly different to that of the air-breathing adult. Internal PCO₂ in the haemolymph of aquatic dragonfly nymphs was variable (~0.5-1.5 kPa), but higher than the crayfish, and could be reduced by hyperventilation during hypoxia exposure. Thus, the CO₂ content of dragonfly nymph blood appears to be higher than that recorded from other water-breathing animals, suggesting that their re-invasion of the aquatic environment and unusual gas exchange strategy separates them from ancestrally water-breathing animals.

A4.7 BIOMINERALIZATION IN THE SEA URCHIN LARVA: FROM ASSEMBLY AND DEPOSITION IN SOFT TISSUES TO FORMATION OF THE CRYSTALLINE SKELETAL MATERIAL

📅 THURSDAY 6 JULY, 2017 ⌚ 13:50

👤 LIA ADDADI (WEIZMANN INSTITUTE OF SCIENCE, ISRAEL), NETTA VIDAUSKY (WEIZMANN INSTITUTE OF SCIENCE, ISRAEL), KEREN KAHIL (WEIZMANN INSTITUTE OF SCIENCE, ISRAEL), STEVE WEINER (WEIZMANN INSTITUTE OF SCIENCE, ISRAEL)

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A major challenge in biomineralization is to determine the pathways by which ions are transferred from external sources to the mineralization site. In sea urchin larvae, we reconstructed a pathway that carries calcium from sea water through the cells to the calcitic spicules. Sea water with its calcium penetrates first into the body cavity, and from there is incorporated into the spicule-forming cells (PMCs) by non-specific endocytosis. Vesicles and vacuoles, some of which are interconnected to form large networks inside the PMCs, have openings through the plasma membrane to the sea water containing body cavity. The vesicles have compositions ranging from essentially identical to sea water, to calcium-enriched, and to

sodium poor compositions. Calcium carbonate is initially deposited as amorphous 20–40 nm particles in some of the vesicles. From there the amorphous calcium carbonate particles are transferred to the site of spicule growth, where transformation into calcite occurs by nucleation from one particle to the next in a complex network. Calcium carbonate particles are also observed in the epithelial cells and in filopodial networks crossing the body cavity, although their fate is yet unknown. A calcium pathway involving direct, non-specific sea water endocytosis was observed also in foraminifera, and may be widespread among organisms of other phyla.

A4.8 CORAL CALCIFYING FLUID pH IS MODULATED BY SEAWATER CARBONATE CHEMISTRY NOT SOLELY SEAWATER pH

THURSDAY 6 JULY, 2017 14:30

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Reef coral calcification depends on regulation of pH in the internal calcifying fluid in which the coral skeleton forms. However, little is known about calcifying fluid pH (pH_{CF}) regulation, despite its importance in determining the response of corals to ocean acidification. We conducted an investigation into the response of pH_{CF} in the reef coral *Stylophora pistillata* in seawater maintained at constant pH with manipulated carbonate chemistry to alter dissolved inorganic carbon (DIC) concentration, and therefore total alkalinity (A_T). We also investigated the intracellular pH of calcifying cells, photosynthesis, respiration, and calcification rates under the same conditions. Our results show that despite constant pH in the surrounding seawater, pH_{CF} is sensitive to shifts in carbonate chemistry associated with changes in [DIC] and A_T , revealing that seawater pH is not the sole driver of pH_{CF} . Notably, when we synthesize our results with published data, we identify linear relationships of pH_{CF} with the seawater [DIC]/[H⁺] ratio, A_T /[H⁺] ratio, and [CO₃²⁻]. Our findings contribute new insight into the mechanisms determining the sensitivity of coral calcification to changes in seawater carbonate chemistry, which is needed for predicting effects of environmental change on coral reefs and for robust interpretations of isotopic paleoenvironmental records in coral skeletons.

A4.9 SODIUM CALCIUM EXCHANGER (NCX) IN CORAL: A POTENTIAL ROLE IN CALCIFICATION

THURSDAY 6 JULY, 2017 14:45

MEGAN E BARRON (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNIVERSITY OF CALIFORNIA SAN DIEGO, UNITED STATES), ANGUS B THIES (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNIVERSITY OF CALIFORNIA SAN DIEGO, UNITED STATES), AMRO HAMDOUN (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNIVERSITY OF CALIFORNIA SAN DIEGO, UNITED STATES), JOSE A ESPINOZA (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNIVERSITY OF CALIFORNIA SAN DIEGO, UNITED STATES), MARTIN TRESGUERRES (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNIVERSITY OF CALIFORNIA SAN DIEGO, UNITED STATES)

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The mechanisms underlying calcification in corals are largely unknown. It has not yet been determined how Ca²⁺, an essential component of corals' CaCO₃ skeletons, is delivered to the subcalicoblastic medium (SCM), the site of calcification. A potential candidate for Ca²⁺ transport is the Sodium Calcium Exchanger (NCX), an antiporter membrane protein that removes one Ca²⁺ ion from cells in exchange for three Na⁺ ions from the external medium. In mammals, different NCX isoforms participate in diverse functions, including general Ca²⁺ homeostasis, muscle contraction, and also bone calcification by osteoblasts. The physiological role of NCX isoforms is largely determined by their subcellular localization. We identified mRNA transcripts for two isoforms of NCX: NCX1 and NCX3, from the stony coral *Acropora yongei*. Sequencing indicates that the NCX3 isoform undergoes alternative splicing to produce at least four distinct proteins. Immunolocalization using custom antibodies designed against an NCX1 sequence from *Acropora digitifera*, revealed that NCX1 was abundant in the coral aboral ectoderm tissue of *A. yongei*, in both calicoblastic (calcifying) and desmocyte cells. The next essential step to elucidate NCX functions in coral is to determine its subcellular localization: presence in the basolateral membrane would suggest a role in removing Ca²⁺, toxic at high concentrations, from the tissue, whereas localization in the apical membrane would indicate a role in delivering Ca²⁺ to the SCM for calcification. Characterization of ion transporters in coral is key to improving mechanistic models of calcification and predicting responses to environmental stressors such as ocean acidification.

A4.10 SYMBIONT PHOTOSYNTHESIS IN GIANT CLAMS IS STRONGLY PROMOTED BY HOST H⁺-TRANSPORT

📅 THURSDAY 6 JULY, 2017 ⌚ 15:00

👤 ERIC J ARMSTRONG (UNIVERSITY OF CALIFORNIA BERKELEY, UNITED STATES), JONATHON H STILLMAN (UNIVERSITY OF CALIFORNIA BERKELEY, UNITED STATES), MARTIN TRESGUERRES (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNITED STATES)

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Giant clams (genus *Tridacna*) are the largest living bivalves and, like reef-building corals, secrete massive calcitic skeletons and host symbiotic algae (zooxanthellae) from which they obtain a significant portion of their respiratory carbon supply. However, both processes (biomineralization and algal photosynthesis) require mechanisms for carbon concentration. In corals, vacuolar-type H⁺-ATPases (VHAs) have been shown to acidify the algal symbiosome, increasing symbiont photosynthesis. Because giant clams occupy similar habitats and perform similar biochemical functions to corals, we hypothesized that VHAs may play analogous roles in tridacnid tissues. We assayed for the presence, and investigated the potential functional roles of VHAs within various tissues of the small giant clam (*Tridacna maxima*). VHAs were present in all tissues assayed (gill, zooxanthellate byssal mantle, and algae-bearing siphonal mantle), but were ~3x more abundant in siphonal mantle where they were located in close proximity to algae-bearing tubules and sites of calcification. Further, VHA contribution to algal photosynthesis was substantial, with inhibition of VHAs reducing symbiont photosynthetic production by nearly 50% in vitro. Algal productivity in light-exposed mantle increased overall energy turn-over by ~100%, and therefore VHA activity likely confers strong ecological and energetic benefits to the host clam. These results confirm the presence of VHAs in tridacnid clams and suggest that, like in corals, they play an important functional role in algal energy generation, potentially sustaining massive growth and permitting the evolution of gigantism in the *Tridacna* lineage.

A4.11 MECHANISMS OF BIOMINERALISATION IN THE MUSSEL: WHAT WE KNOW AND WHAT WE STILL NEED TO FIND OUT

📅 THURSDAY 6 JULY, 2017 ⌚ 15:45

👤 SUSAN FITZER (UNIVERSITY OF GLASGOW, UNITED KINGDOM)

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Global climate change threatens the oceans as anthropogenic carbon dioxide causes ocean acidification and warming. The amount of carbonate available in the oceans under ocean acidification will be reduced. Calcifying organisms, such as shellfish, are those at most risk from such ocean acidification, as carbonate is vital in the biomineralisation of their calcium carbonate protective shells. There is uncertainty behind the mechanisms of carbonate uptake, carbonate can be sourced through the environment as dissolved inorganic carbon as carbonate or hydrogen carbonate. Molluscs can also incorporate metabolically derived carbon through CO₂ entering the extrapallial fluid by diffusion from the mantle tissue. The ability to differentiate between these carbon sources is a crucial gap in knowledge for defining the pathways involved in biomineral shell production. This study examines the mineral specific carbon source and route for shell production or 'biomineralisation pathway' under ocean acidification and warming for three mollusc species. Here I present data for the mussel, *Mytilus edulis*, which suggests that ocean acidification can implement a fundamental shift in the carbon source utilised for biomineralisation. This research highlights the reduced capacity of mussels to biomineralise, sourcing carbon in the form of carbonate rather than tissue bicarbonate produced via protein activity. Projections for ocean acidification and reduced carbonate saturation levels may prove detrimental to mussel shell production. Knowledge of the mineral specific biomineralisation pathways, in combination with the shell physical properties, will enable accurate predictions of the vulnerability of these mollusc species to future climate change.

A4.12 BUILDING SHELLS IN A CHANGING WORLD

📅 THURSDAY 6 JULY, 2017 ⌚ 16:15

👤 MELODY S CLARK (BRITISH ANTARCTIC SURVEY, UNITED KINGDOM)

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70% of our planet is covered by oceans, which host an incredible range of biodiversity, and a significant source of food for the World's growing population. Unfortunately, like the rest of the planet, this blue world is increasingly affected by global change; it is becoming warmer and is acidifying. We know this will affect the endemic fauna, but how will these blue ecosystems change? Marine invertebrates, such as molluscs are suggested to be particularly at risk. Although, we know that molluscs have the capacity to modify the shape and thickness of their shell depending on their habitat, as well as in response to other environmental factors such as wave action, iceberg activity and also the presence of predators, we do not know exactly how they build their shells. What are the molecular pathways involved and the costs of building a shell? How does shell structure vary in different

habitats? What is the interplay between genetics and environment? What genetic variability is there in different populations: can we select for more robust shell builders? This talk, on behalf of 14 young researchers in the EU Marie Curie funded CACHE (Calcium in a Changing Environment) ITN (grant: 605051), will present an overview of the results from the past 3 years from where we conducted research into all these questions using four commercially important molluscs: the Pacific oyster, the blue mussel, king scallops and soft-shelled clams and also evaluated shell waste remediation and the consequences of climate change for the shellfish industry.

A4.13 CACHE - CALCIUM IN A CHANGING ENVIRONMENT

📅 THURSDAY 6 JULY, 2017 ⌚ 16:30

👤 KATI MICHALEK (SCOTTISH ASSOCIATION FOR MARINE SCIENCE, UNITED KINGDOM), ALEXANDER VENTURA (UNIVERSITY OF GOTHENBURG, SWEDEN), NADÈGE ZAGHDOUDI-ALLAN (CCMAR, PORTUGAL), LUCA TELESCA (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), TEJASWI YARRA (BRITISH ANTARCTIC SURVEY, UNITED KINGDOM), TRYSTAN SANDERS (GEOMAR, GERMANY), JAISON ARIVALAGAN (NATIONAL HISTORY MUSEUM, FRANCE), KIRSIKKA SILLANPÄÄ (UNIVERSITY OF GOTHENBURG, SWEDEN), KIRTI RAMESH (GEOMAR, GERMANY), DAVID VENDRAMI (UNIVERSITY OF BIELEFELD, GERMANY), PHOEBE STEWART-SINCLAIR (SCOTTISH ASSOCIATION FOR MARINE SCIENCE, UNITED KINGDOM), JAMES MORRIS (ROYAL BELGIUM INSTITUTE OF NATURAL SCIENCES, BELGIUM), YAN WANG-DUFFORT (ROYAL BELGIUM INSTITUTE OF NATURAL SCIENCES, BELGIUM), MICHELE DE NOIA (UNIVERSITY OF BIELEFELD, GERMANY), CARLOS CAURCEL (UNIVERSITY OF EDINBURGH, UNITED KINGDOM)

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With this Pecha Kucha event, we are delighted to summarise present-day research in the field of mollusc shell production and biotechnology in a changing world. Launched in November 2013 and running for 4 years, the Marie Curie Initial Training Network CACHE (Calcium in a Changing Environment; grant#605051) brought together 15 PhD students and early researchers from all over the world. CACHE comprises 10 partners from 6 European countries, including SMEs and a shellfish consultancy, ensuring multi-disciplinary and applied research. Using mollusc species of commercial interest (Pacific oysters, blue mussels, king scallops and soft-shelled clams) we tackle complex biological questions while forming the bridge between academic research and industrial implementation. Speed presentations by our young researchers will cover questions ranging from how a mollusc shell is produced, differences in shell production in varying habitats, potential to select for more robust shell builders, as well as the use of shell waste for biotechnological applications, to name only some of the work. Therefore this CACHE Pecha Kucha will provide the full picture necessary to understand how shell building and mollusc cultivation might be impacted by future climate conditions.

A4.14 SCOTTISH BLUE MUSSELS - EVIDENCE FOR CHANGE DOWN THE CULTIVATION ROPE

📅 THURSDAY 6 JULY, 2017 ⌚ 16:45

👤 KATI MICHALEK (SCOTTISH ASSOCIATION FOR MARINE SCIENCE, UNITED KINGDOM), KIM S LAST (SCOTTISH ASSOCIATION FOR MARINE SCIENCE, UNITED KINGDOM), THOMAS A WILDING (SCOTTISH ASSOCIATION FOR MARINE SCIENCE, UNITED KINGDOM), DAVID GREEN (SCOTTISH ASSOCIATION FOR MARINE SCIENCE, UNITED KINGDOM), JOSEPH I HOFFMAN (UNIVERSITY OF BIELEFELD, GERMANY)

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The Scottish blue mussel industry reached record production values in 2014 of 7.7k tonnes being worth £9.2m, supporting the local economy and employment. Mussel cultivation relies on natural spat fall in habitats with pronounced environmental variability, both on small and large temporal and spatial scales. We monitored fluctuations in key environmental drivers (temperature, salinity, food availability, seawater carbonate chemistry) and the corresponding quality of the cultured mussels (condition index, meat yield) over one year and at different depth of cultivation at a mussel farm on the west coast of Scotland, UK. Whilst both the environment and mussel product quality varied seasonally as expected, the most dramatic responses were with depth i.e. down the length of a mussel cultivation rope. The range in water salinity of near-surface grown mussels varied from 1.8 to 29.2 PSU within days, with far less variation for deeper grown mussels (27.0 ± 3.7 PSU at 7m). Seasonal and vertical fluctuations in temperature, salinity and food availability, in particular, were closely associated with the mussels' product quality with maximum meat yields achieved in the top meters of the water column and during summer months. In addition, the shape and strength of the mussels shells were found to differ down a cultivation rope, reflecting differences in species distribution (more fragile shelled *M. trossulus* in surface waters), stocking density (decreasing with depth) and prevailing habitat conditions (e.g. lower salinity in surface waters), allowing for the implementation of such data for farm management practices.

A4.15 ADAPTATION POTENTIAL TO OCEAN ACIDIFICATION IN THE BLUE MUSSEL *MYTILUS EDULIS*

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

👤 ALEXANDER VENTURA (UNIVERSITY OF GOTHENBURG, SWEDEN)

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Ocean acidification (OA) has the potential to impact marine organisms. Species producing calcium carbonate can be particularly sensitive. These include bivalve molluscs, many of which, such as the blue mussel (*Mytilus edulis*), are of high ecological and economic value. Populations' acclimatisation and adaptation potentials are often difficult to investigate and comprehend, especially for organisms with long generation times. However these need to be accounted for and are crucial for the understanding of environmental change impacts. The overall aim of the present work is to investigate the adaptation potential to OA in the blue mussel *M. edulis*. The project is divided into three chapters: 1) Identification of seawater acidity tolerance threshold in *M. edulis* larvae (Ventura et al., 2016). Our results suggest considerable phenotypic plasticity of larvae within natural range of pH variability which may represent an advantage for coping with OA. 2) Investigation of heritability and additive genetic variance associated with phenotypic traits affected by more acidic waters (ongoing work). 3) Analysis of interaction and potential evolutionary trade-offs between higher seawater acidity and other environmental stressors (i.e. decreasing seawater salinity) (ongoing work).

A4.16 AMTs IN INVERTEBRATES: NEW PLAYERS IN AMMONIA TRANSPORT AND ACID-BASE REGULATION

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

👤 DIRK WEIHRAUCH (UNIVERSITY OF MANITOBA, CANADA), AIDA ADLIMOGHADDAM (UNIVERSITY OF MANITOBA, CANADA), ANDREW DONINI (YORK UNIVERSITY, CANADA)

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Ammonia transporters (AMTs) are the key transporters for the uptake of ammonia from the soil in plants (AMTs). They are distantly related to the Rh-glycoproteins however, in contrast to the Rh-proteins, they have not been shown to be expressed in vertebrate animals. Most interestingly, numerous genome and transcriptome projects revealed that AMTs are indeed expressed in invertebrates. Their actual role here needs to be examined. A study by the Zwiebel group (2014) suggested that an AMT from *Anopheles gambiae*, when expressed in oocytes, promotes a NH_4^+ transport. Moreover, most recent physiological studies by the Donini group on *Aedes aegypti* larvae showed a strong decrease of the ammonia excretion rates in the excretory anal papillae when the AMT was knocked down. In the marine polychaete *Eurythoe complanata*, three AMTs were identified, all of them higher expressed in the gills when compared to the main body. In fact, branchial AMT4 exhibited ca. 8.5-times higher transcript levels when compared to the alpha subunit of the Na^+/K^+ -ATPase. AMT function is however not clear-cut. For instance, out of the four AMTs expressed in *C. elegans* only one responded to elevated environmental ammonia levels. By contrast, exposure

to a high or low environmental pH triggered changes in transcript levels in all AMTs expressed in the nematode. Consequently, a role in acid-base regulation is assumed.

A4.17 RAD SEQUENCING RESOLVES FINE-SCALE POPULATION STRUCTURE IN A BENTHIC INVERTEBRATE: IMPLICATIONS FOR UNDERSTANDING PHENOTYPIC PLASTICITY

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

👤 DAVID LJ VENDRAMI (UNIVERSITY OF BIELEFELD, GERMANY), LUCA TELESCA (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), HANNAH WEIGAND (UNIVERSITY OF DUISBURG-ESSEN, GERMANY), MARTINA WEISS (UNIVERSITY OF DUISBURG-ESSEN, GERMANY), KATIE FAWCETT (UNIVERSITY OF BIELEFELD, GERMANY), KATRIN LEHMAN (UNIVERSITY OF BIELEFELD, GERMANY), MELODY S CLARK (BRITISH ANTARCTIC SURVEY, UNITED KINGDOM), FLORIAN LEESE (UNIVERSITY OF DUISBURG-ESSEN, GERMANY), CARRIE MCMINN (AGRI-FOOD AND BIOSCIENCES INSTITUTE, UNITED KINGDOM), HEATHER MOORE (AGRI-FOOD AND BIOSCIENCES INSTITUTE, UNITED KINGDOM), JOSEPH I HOFFMAN (UNIVERSITY OF BIELEFELD, GERMANY)

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The field of molecular ecology is transitioning from the use of small panels of classical genetic markers such as microsatellites to much larger panels of SNPs generated by approaches like RAD sequencing. However, few empirical studies have directly compared the ability of these methods to resolve population structure. This could have implications for understanding phenotypic plasticity, as many previous studies of natural populations may have lacked the power to detect genetic differences, especially over micro-geographic scales. We therefore compared the ability of microsatellites and RAD sequencing to resolve fine-scale population structure in a commercially important benthic invertebrate by genotyping great scallops (*Pecten maximus*) from nine populations around Northern Ireland at 13 microsatellites and 10,539 SNPs. The shells were then subjected to morphometric and colour analysis in order to compare patterns of phenotypic and genetic variation. We found that RAD sequencing was superior at resolving population structure, yielding higher F_{st} values and support for two distinct genetic clusters whereas only one cluster could be detected in a Bayesian analysis of the microsatellite dataset. Furthermore, appreciable phenotypic variation was observed in size-independent shell shape and colouration, including among localities that could not be distinguished from one another genetically, providing support for the notion that these traits are phenotypically plastic. Taken together, our results suggest that RAD sequencing is a powerful approach for studying both population structure and phenotypic plasticity in natural populations.

A4.18 INSIGHTS INTO HOW BIOMINERALIZATION IS REGULATED IN THE MEDITERRANEAN AND BLUE MUSSEL

WEDNESDAY 5 JULY, 2017 POSTER SESSION

NADEGE ZAGHDOUDI-ALLAN (CENTER OF MARINE SCIENCES UNIVERISTY OF THE ALGARVE, PORTUGAL), DEBORAH POWER (CENTER OF MARINE SCIENCES UNIVERISTY OF THE ALGARVE, PORTUGAL), KRISTINA SUNDELL (GOTHENBURG UNIVERSITY, SWEDEN)

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Biom mineralization is the physiological process through which living organisms generate mineralized structures. These structures can have multiple functions, including tissue support, protection from the external environment, feeding and sensing. Understanding the underlying mechanisms of biomineralization and the effects of external stress are vital for the maintenance of food security, ecological stability and a sustainable shellfish industry. In this study, the biomineralization process in the Mediterranean and Blue mussel was investigated using a suite of techniques and focused on the mantle tissue. Transcriptomics was used to determine mantle genes regulated along the shell from tip to tip. Histology was carried out to look at the structure across the mantle tissue. Electrophysiology was used to study the permeability of the mantle tissue. Results from these studies showed that the mantle tissue is a structurally and functionally complex organ. Its structure and function differ across the different regions of the tissue and thus the shell. Our results give insight into the mechanisms used by these animals to form their outer shell.

A4.19 TRANSFORMATION AND TRANSPORTATION OF SULFUR COMPOUNDS IN GILLS OF HYDROTHERMAL VENT CRAB *XENOGRAPSUS TESTUDINATUS* NEAR KUISHAN ISLAND, TAIWAN

WEDNESDAY 5 JULY, 2017 POSTER SESSION

PEI-HSUAN CHOU (DEPARTMENT OF LIFE SCIENCE, NATIONAL TAIWAN NORMAL UNIVERSITY, TAIWAN), PEI-CHEN HWANG (MARINE RESEARCH STATION INSTITUTE OF CELLULAR AND ORGANISMIC BIOLOGY ACADEMIA SINICA, TAIWAN), POU-LONG KUAN (MARINE RESEARCH STATION INSTITUTE OF CELLULAR AND ORGANISMIC BIOLOGY ACADEMIA SINICA, TAIWAN), YI-TA SHAO (INSTITUTE OF MARINE BIOLOGY, NATIONAL TAIWAN OCEAN UNIVERSITY, TAIWAN), GUAN-CHUNG WU (DEPARTMENT OF AQUACULTURE, NATIONAL TAIWAN OCEAN UNIVERSITY, TAIWAN), YUNG-CHE TSENG (MARINE RESEARCH STATION INSTITUTE OF CELLULAR AND ORGANISMIC BIOLOGY ACADEMIA SINICA, TAIWAN)

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Different from other marine ecosystems, the shallow hydrothermal vents system of Kueishan Island off the coast of Taiwan contains high sulfur compounds due to the release of sulfur particles from discharging leak. And highly sulfidic conditions would inhibit cellular aerobic respiration in organisms. To survive in this extreme habitats, vent associated organisms, such as endemic crab species *Xenograpsus testudinatus*, may show a range of physiological feature to cope with toxic environment. In this study, we found the presence of hypotaurine and thiotaurine in their hemolymph, and transcript expressions of taurine transporter (TAUT) in the 3rd and 5th gill pair are significantly higher in native sulfidic environment than the none sulfur-treated counterparts. These results inferred that *X. testudinatus* gills are capable to catabolize hypotaurine and S²⁻ thus synthesize thiotaurine to reduce cellular toxicity. In addition, relative highly expressions of sulfur transport-related transcript, solute carrier family 26A11 (SLC26A11), was found in native individuals as well. It implies that sulfide may be oxidized to sulfate then transported by SLC26A11 in gill epithelium, and sulfur-induced Na⁺-K⁺-ATPase upregulation may be involved in cellular homeostasis. Accordingly, *X. testudinatus* have evolved an efficient sulfur detoxification mechanism in gill epithelium to minimize continuously toxic stress under challenging hydrothermal vent system.

A4.20 BLUE MUSSEL SHELL SHAPE PLASTICITY AND NATURAL ENVIRONMENTS: A QUANTITATIVE APPROACH

WEDNESDAY 5 JULY, 2017 POSTER SESSION

LUCA TELESCA (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), KATI MICHALEK (SCOTTISH ASSOCIATION FOR MARINE SCIENCE, UNITED KINGDOM), TRYSTAN SANDERS (GEOMAR HELMHOLTZ CENTRE FOR OCEAN RESEARCH, GERMANY), LLOYD PECK (BRITISH ANTARCTIC SURVEY, UNITED KINGDOM), ELIZABETH M HARPER (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM)

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Morphological variability represents an important direct response of organisms to selecting environments. We show how geometric morphometrics and generalized additive mixed models (GAMMs) can provide a better understanding of the environmental responsiveness of *Mytilus edulis* form. Morphology of wild and cultured blue mussels was accurately quantified and significant relationships between shell shape and key environmental parameters were identified. Evident trends of mean shape with physical descriptors (water temperature and salinity), depending on the shell traits considered, were found; while, food supply (chlorophyll-a concentration) showed a marked effect on shapes heterogeneity. The observed convergence of *M. edulis* morphologies to adverse conditions, in different habitats, identified mussel shape variation as a marked response to environmental stressors and explained how local factors can produce the development of a wide range of forms. Our findings illustrate that the use of novel tools and complementary systems can allow to identify fine-scale shape clines and reveal patterns in bivalve plasticity, in order to understand morphological responses to different habitats. We show how shell shape can represent a powerful indicator to better understand response of marine communities and predict their changes in a rapidly changing environment.

A4.21 CROSSED-LAMELLAR MICROSTRUCTURE OF MOLLUSK SHELLS, NEW INSPIRATION OF BIOMIMETIC MATERIAL

WEDNESDAY 5 JULY, 2017 POSTER SESSION

YAN WANG-DUFFORT (ROYAL BELGIAN INSTITUTE OF NATURAL SCIENCES, BELGIUM), THIERRY BACKELJAU (ROYAL BELGIAN INSTITUTE OF NATURAL SCIENCES, BELGIUM), JAMES MORRIS (ROYAL BELGIAN INSTITUTE OF NATURAL SCIENCES, BELGIUM), GAUTHIER CHAPPELLE (ROYAL BELGIAN INSTITUTE OF NATURAL SCIENCES, BELGIUM)

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Mollusk shells represent an important portion of the large family of biomaterials. By studying the shell microstructures, we gain a better understanding of how mollusks have developed exceptionally strong protective shells. The nacreous layer and the crossed-lamellar (CL) layer have been shown to be several orders of magnitude stronger than their geological counterparts, aragonite. A large number of proteins are involved in the control of biogenic mineral formation. The CL layer contains much less organic matter compared with in nacre, less energy is required for protein synthesis. This low energy-consuming layer is the most common type of mollusk shell microstructure.

We are interested in the comparison of hierarchical architectures and crystallographic form in CL layer and nacreous layer, for getting a better insight of biogenic mineral evolution. The potential use of our understanding of the microstructure in novel bio-inspired and biomimetic applications may be feasible for developing material with high fracture toughness and low cost.

A4.22 THE EFFECT OF CLIMATE CHANGE ON THE PHYSIOLOGY OF THE LOUISIANA RED SWAMP CRAYFISH (*PROCAMBARUS CLARKII*)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

ASHLEY TRIPP (UNIVERSITY OF MANITOBA, CANADA), ALEX QUIJADA-RODRIGUEZ (UNIVERSITY OF MANITOBA, CANADA), GARETT JP ALLEN (UNIVERSITY OF MANITOBA, CANADA), GWANGSEOK R YOON (UNIVERSITY OF MANITOBA, CANADA), DIRK WEIHRACH (UNIVERSITY OF MANITOBA, CANADA)

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As a direct result of anthropogenic carbon emissions ocean and freshwater acidity is increasing, presenting a risk to important ecological and economic species such as the freshwater crayfish, *Procambarus clarkii*. Indigenous to Louisiana and found in the Atchafalaya River Basin approximately 181 million dollars in crayfish are harvested and commercially sold annually from Louisiana alone, making it the largest contributor to the crayfish industry within the United States. Although farmed crayfish populations can be protected from the damaging effects of acidification, the river basin populations, representing 90% of the export, are at risk to changing water conditions. Increasing temperature has been well studied as a factor of climate change but the combined effect of temperature and increasing pCO₂ are not well

described, especially in understudied freshwater systems. In this study, changes in whole animal ammonia excretion, hemolymph chemistry and whole body metabolic rate were investigated, under control conditions (pH=8.04, Temp=24.1°C, pCO₂=92.17 Pa) and a simulation of a potential year 2100 (pH=7.53, Temp=28.18°C, pCO₂=272.17 Pa). Ammonia excretion rates of animals acclimated to elevated pCO₂ for 7 days did not change but there was a significant reduction of hemolymph pH, as compared to controls (N=8) suggesting an acidosis and potentially hampered secretion of acid-base equivalents. The average mass specific metabolic rate was significantly higher in elevated pCO₂ exposed crayfish (202.2 mg O₂/Kg/Hr) over controls (87.3 mg O₂/Kg/Hr) (N=8 and N=6, respectively).

A4.23 POPULATION GENETIC STRUCTURE OF THE SOFT SHELL CLAM, *MYA ARENARIA*, ALONG A EUROPEAN LATITUDINAL GRADIENT

WEDNESDAY 5 JULY, 2017 POSTER SESSION

MICHELE DE NOIA (UNIVERSITY OF BIELEFELD, GERMANY), DAVID D VENDRAMI (UNIVERSITY OF BIELEFELD, GERMANY), LUCA TELESKA (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), ELIZABETH HARPER (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), JOSEPH HOFFMANN (UNIVERSITY OF BIELEFELD, GERMANY)

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Mya arenaria (Linnaeus 1758) is a soft shell clam widely distributed across the North Hemisphere, along the Atlantic and Pacific coasts. However, little is known about the population structure of this species, particularly in Europe where it re-established itself during the early Pliocene. We therefore collected a total of 257 clams from 9 sites across a European latitudinal gradient, spanning the Mediterranean Sea to Scotland. All of the individuals were genotyped at 12 microsatellites and the COI mitochondrial gene was also amplified for a subset of randomly selected samples to verify that the specimens were of the correct species. We found evidence of strong population structure that is also reflected to a certain extent in the morphology of the shells. We discuss the implications of our results for understanding the population structure of clams and related species in relation to potential ecological and environmental drivers.

A4.24 MOLLUSC SHELLS ARE A VALUABLE BIOMATERIAL, NOT A NUISANCE WASTE PRODUCT OF THE AQUACULTURE INDUSTRY

WEDNESDAY 5 JULY, 2017 POSTER SESSION

JAMES PETER MORRIS (ROYAL BELGIAN INSTITUTE OF NATURAL SCIENCES, BELGIUM), GAUTHIER CHAPPELLE (INDEPENDENT RESEARCHER, BELGIUM), THIERRY BACKELJAU (ROYAL BELGIAN INSTITUTE OF NATURAL SCIENCES, BELGIUM)

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Shelled molluscs are an important component of the rapidly growing global aquaculture sector. In a changing environment, and under increasing demand, future sustainability is a key consideration for aquaculture, as a whole, moving forwards. One regularly overlooked aspect of the molluscaquaculture and seafood industries is the generation of calcium carbonate shells as a by-product. Typically, shells are viewed by these industries as a nuisance waste product and disposed of, at cost, in landfill. In contrast, calcium carbonate is mined in vast quantities in the form of limestone for various applications. As part of the Marie Curie CACHE network (<http://www.cache-itn.eu>), this project explores existing and novel uses of shells as a by-product of the aquaculture industry. Analysis shows that, with minimal processing, shells can be turned into a valuable resource for a variety of applications, from simple aggregates to urban bio-filter substrates. Further, taking an ecosystem services approach, shells have inherent value within the marine system, and co-ordinated efforts to return shells to the marine environment following food processing may provide a highly sustainable avenue for shell reuse. In such cases, whole shells can provide the foundation for biogenic reef formation and restoration, and crushed or powdered shells may have value in future aquaculture-based carbon trading systems.

A4.25 CALCIUM TRANSPORT IN THE OUTER MANTLE EPITHELIUM OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

KIRSIKKA SILLANPÄÄ (DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES UNIVERSITY OF GOTHENBURG, SWEDEN), KRISTINA SUNDELL (DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES UNIVERSITY OF GOTHENBURG, SWEDEN)

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Bivalves are a class of molluscs characterised by their protective calcium carbonate shell. To build and maintain the shell, marine bivalves, such as *Crassostrea gigas*, need to take up calcium from the seawater. In order to be used as a shell component, calcium is transported to the area of shell growth. The final part of the transport occurs across the outer mantle epithelium (OME), separating the shell from the rest of the animal. In theory, calcium could be transported across the OME either intra- or paracellularly as free, ionic calcium, bound to organic or inorganic ligands, bound to proteins and/or within vesicles or hemolymph cells. Ionic Ca^{2+} has been found to be the main calcium species in the hemolymph of *C. gigas*. Therefore the aim of this study was to investigate the ionic Ca^{2+} transport systems through the OME of this species. The Ca^{2+} transport across the epithelium can be executed through calcium channels, plasma membrane Ca^{2+} -ATPases (PMCA) and/or Na^+ / Ca^{2+} -exchangers. A general ATPase inhibitor, Vanadate and specific PMCA inhibitors, Caloxins, were used to assess the importance of PMCA-like transporters in Ca^{2+} transport through the OME. *In vivo* Using chamber methodology together with radiolabelled ^{45}Ca was used to measure the Ca^{2+} transport rate across the OME with and without inhibitors. Vanadate and Caloxin-1c2 inhibited Ca^{2+} transport when added from the shell side. This indicates that PMCA-like transporters are situated in the shell facing membrane of the OME and are participating in the Ca^{2+} transport across the OME of *Crassostrea gigas*.

A5 OSMOREGULATION AND ACID-BASE BALANCE IN AQUATIC ORGANISMS

ORGANISED BY: KEVIN BRIX (UNIVERSITY OF MIAMI, UNITED STATES), ANDREW ESBAUGH (UNIVERSITY OF TEXAS, UNITED STATES) AND MARTIN TRESGUERRES (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNITED STATES)

SESSION SPONSORED BY: LOLIGO SYSTEMS

A5.1 CARBONIC ANHYDRASE IN THE IONOCYTES OF THE FISH GILL: RESPONSES TO ACID-BASE CHALLENGES

📅 MONDAY 3 JULY, 2017 ⌚ 09:00

👤 KATHLEEN GILMOUR (UNIVERSITY OF OTTAWA, CANADA)

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The 'morphological model' for acid-base regulation in freshwater teleost fish was proposed to explain differences in the morphology of the gill epithelium that were detected in response to acid-base disturbances. In particular, the surface area of the gill epithelium occupied by mitochondrion-rich cells (MRCs) decreased in rainbow trout experiencing systemic acidosis, and increased in fish experiencing systemic alkalosis. At the time the model was proposed, the diversity of freshwater MRCs or ionocytes had not yet been recognized - MRCs were considered to be freshwater 'chloride cells', base-secreting cells that were responsible for Cl⁻ uptake. Thus, decreasing chloride cell surface area would decrease HCO₃⁻ loss during acidosis and increased chloride cell surface area would increase base excretion during alkalosis, providing a morphological component to acid-base compensatory responses. Current work exploring the diversity of ionocytes in the gill of freshwater trout, the distribution of carbonic anhydrase in these ionocytes, and the regulation of carbonic anhydrase expression in response to acid-base disturbances is providing additional insight into the 'morphological model' of acid-base regulation in rainbow trout.

A5.2 EXTENDING THE DOGFISH MODEL OF CO₂ EXCRETION TO THE GILLS AND BLOOD OF OTHER CHONDRICHTHYAN FISHES

📅 MONDAY 3 JULY, 2017 ⌚ 09:40

👤 OLIVIA J MCMILLAN (UNIVERSITY OF BRITISH COLUMBIA, CANADA), ANGELINA M DICHIERA (UNIVERSITY OF TEXAS AT AUSTIN MARINE SCIENCE INSTITUTE, UNITED STATES), TILL S HARTER (UNIVERSITY OF BRITISH COLUMBIA, CANADA), MICHAEL SACKVILLE (UNIVERSITY OF BRITISH COLUMBIA, CANADA), ANDREW J ESBAUGH (UNIVERSITY OF TEXAS AT AUSTIN MARINE SCIENCE INSTITUTE, UNITED STATES), COLIN J BRAUNER (UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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The enzyme carbonic anhydrase (CA) catalyzes the conversion of H⁺ and HCO₃⁻ to carbon dioxide (CO₂) and H₂O and is essential for CO₂ excretion. In most teleosts, membrane-bound plasma accessible CA (paCA) is lacking in the gills, restricting this process to the red blood cell (RBC). In the dogfish *Squalus suckleyi*, however, the RBC and plasma compartments appear equally important for CO₂ excretion because of paCA at the gill, higher plasma buffering capacity, slower RBC CA and the absence of a plasma CA inhibitor. This model of CO₂ excretion has only been thoroughly investigated in dogfish and this project investigates whether it also applies more broadly to chondrichthyans as a whole. Blood samples were collected from 15 species and assessed for CA characteristics and plasma buffering capacity and gill samples were collected from 6 species and assessed for CA activity associated with the microsomal gill fraction. To address whether this CA is truly plasma-accessible, immunohistochemical techniques were used on fixed gill tissue to visualize the CA orientation. CA characteristics and plasma buffering capacity in blood samples were consistent with those of *S. suckleyi*, and paCA was found in the gills of blacktip reef sharks (*Carcharhinus melanopterus*), Atlantic stingrays (*Dasyatis sabina*) and ocellate river stingrays (*Potamotrygon motoro*). The results of this research suggest that the dogfish model of CO₂ excretion may be representative of chondrichthyan fishes in general and provides important information regarding general patterns of gas exchange and acid-base balance among this phylogenetically diverse group.

A5.3 PREFERENTIAL INTRACELLULAR PH REGULATION IN VERTEBRATES

MONDAY 3 JULY, 2017

09:55

COLIN J BRAUNER (UNIVERSITY OF BRITISH COLUMBIA, CANADA), RYAN B SHARTAU (UNIVERSITY OF BRITISH COLUMBIA, CANADA), DAN W BAKER (VANCOUVER ISLAND UNIVERSITY, CANADA)

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Acid-base regulation is a tightly regulated process and pH disturbances are rapidly corrected to restore homeostasis. In most vertebrates investigated to date, acute (up to 96 h) exposure to elevated environmental CO₂ (hypercarbia) results in a rapid reduction in both blood pH (pH_e) and intracellular pH (pH_i) and pH_i recovers in conjunction with pH_e compensation, called coupled pH regulation. However, in a few species of extremely CO₂ tolerant fishes, such as white sturgeon, severe hypercarbia results in a large, uncompensated reduction in pH_e (in some cases exceeding 1 pH unit); however, remarkably, there is no change in pH_i of heart, brain, liver or muscle, termed preferential pH_i regulation. To investigate the time course of preferential pH_i regulation, we used ³¹P nuclear magnetic resonance (NMR) technology to measure heart pH_i and metabolites in vivo throughout the first 90 minutes of exposure to severe hypercarbia (pCO₂ = 6 kPa) in white sturgeon. Heart pH_i was not significantly reduced at any time (measured every 2.5 minutes) indicating that preferential pH_i is very rapid. Recent studies on 15 other phylogenetically diverse fish species indicate that preferential pH_i regulation may be a relatively widespread pattern of acid-base regulation in CO₂ tolerant fishes and we propose that it represents an important adaptation in the evolution of air-breathing and the transition of vertebrates from water to land.

A5.4 A PARADIGM SHIFT IN VERTEBRATE ACID-BASE REGULATORY STRATEGY: PREFERENTIAL INTRACELLULAR PH REGULATION AS A BROADLY USED STRATEGY OF PH REGULATION AMONGST VERTEBRATES

MONDAY 3 JULY, 2017

10:25

RYAN B SHARTAU (UNIVERSITY OF BRITISH COLUMBIA, CANADA), COLIN J BRAUNER (UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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Acid-base homeostasis is tightly regulated in vertebrates as small changes in pH alter protein charge and function, ultimately reducing whole animal performance. In some environments vertebrates may be subjected to high CO₂ (hypercarbia) partial pressure (PCO₂), leading to a severe respiratory acidosis. Typically, blood pH (pH_e) is compensated, which is coupled to tissue pH (pH_i) regulation; however, this process appears to be limited to a PCO₂ 1 pH unit) and can tolerate PCO₂ >3 kPa; there is indirect evidence for ppH_i in embryonic amniotes. It is uncertain whether ppH_i represents a common vertebrate strategy in tolerating high CO₂ environments. We hypothesize that vertebrates use ppH_i to maintain pH homeostasis during severe acute acid-base disturbances. To address

this, we investigated the presence or absence of ppH_i in fishes and embryonic amniotes during exposure to severe acute hypercarbia by measuring pH_e and pH_i of heart, brain, liver and muscle following CO₂ exposure. Our results suggest ppH_i is used by >20 fishes and two species of amniote embryos. Almost all species investigated fully protect pH_i during acute CO₂ exposure of 6-15 kPa PCO₂ despite severe pH_e reduction. The use of ppH_i in phylogenetically diverse vertebrates represents a markedly different strategy than coupled pH_e/pH_i regulation and we propose that ppH_i is a general strategy of acid-base regulation in embryonic vertebrates that is retained or lost during development.

A5.5 AIR-BREATHING CHANGES THE PATTERN FOR TEMPERATURE INDUCED PH REGULATION IN A BIMODAL BREATHING TELEOST

MONDAY 3 JULY, 2017

10:40

CHRISTIAN DAMSGAARD (AARHUS UNIVERSITY, DENMARK), MIKKEL THY THOMSEN (AARHUS UNIVERSITY, DENMARK), MARK BAYLEY (AARHUS UNIVERSITY, DENMARK), TOBIAS WANG (AARHUS UNIVERSITY, DENMARK)

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It is well-established that ectothermic vertebrates regulate a lower arterial pH when temperature increases. Typically, water-breathers reduce arterial pH by means of altering plasma [HCO₃⁻] when temperature rises, whilst air-breathers rely on ventilatory adjustments to elevate arterial PCO₂. However, little emphasis has been devoted to understand how bimodal breathing vertebrates achieve their temperature-induced pH-regulation. In this context, the air-breathing catfish *Pangasianodon hypophthalmus* represents an interesting possibility to unravel how pH regulation is affected by water versus air-breathing pattern. *P. hypophthalmus* can accommodate all gas exchange by its well-developed gills in normoxic water, but resort to air-breathing by virtue of the swim-bladder when exposed to aquatic hypoxia. We therefore measured acid-base status in *P. hypophthalmus* as temperature rose from 20 to 35°C in either normoxic or severely hypoxic water. In normoxic water, where *P. hypophthalmus* relied entirely on brancial gas exchange, *P. hypophthalmus* exhibited the typical teleost reduction in plasma [HCO₃⁻] and arterial pH when temperature rose. However, when forced to rely air-breathing in severe aquatic hypoxia, the reduction in arterial pH was achieved by a rise in arterial PCO₂. We propose that the rise in PCO₂ reflects a passive consequence of the progressive transition to air-breathing at higher temperatures, and that this response matches the new regulated pH_a, and merely requires small adjustments in plasma [HCO₃⁻]. This is the first experimental documentation of an intraspecific shift in the pattern for temperature-induced pH-regulation, and illustrates a remarkable resemblance to the evolutionary changes in temperature-induced pH-regulation that occurred during terrestrialization of vertebrates.

A5.6 ACID-BASE BALANCE IN THE MAMMALIAN KIDNEY

📅 MONDAY 3 JULY, 2017 ⌚ 14:00

👤 SETH L ALPER (BETH ISRAEL DEACONESS MEDICAL CENTER, UNITED STATES)

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Mammals rely primarily on the kidneys and the lungs to maintain systemic pH of extracellular fluid between 7.35 and 7.45, with intracellular pH generally about 0.1-0.2 pH units lower (or higher in specialized acid-secreting cells). Maintenance of this pH range is essential to neuronal and cardiovascular function, and to homeostasis of enzymatic activity and integrity of signal transduction. pH regulation is also essential for the axial macro- and microheterogeneity of normal epithelial and glandular function. The lungs control exhalation of CO₂ produced by metabolism. The ~2 million nephrons of human kidneys reabsorb nearly all filtered bicarbonate (~4000 mmol per day), and actively excrete the net 50-100 mmol of H⁺ generated by metabolism of dietary sulfate and phosphate. This talk will review the major proton and bicarbonate transporters, their integration in the function of kidney and gut and the pathological consequences of their loss of function secondary to genetic inactivation. Type A acid-secreting intercalated cell dysfunction causes distal renal tubular acidosis (dRTA). Recessive human dRTA has been linked to loss-of-function mutations in the B1 and a4 subunits of the vacuolar H⁺-ATPase. Recessive and dominant dRTA have been linked to distinct loss-of-function mutations in the SLC4A1 Cl⁻/HCO₃⁻ exchanger. Loss-of-function mutations in NBCe1/SLC4A4 cause recessive proximal tubular acidosis, and loss-of-function mutations in CA2 (carbonic anhydrase 2) cause mixed proximal-distal tubular acidosis. Intracellular pH regulation is altered in cancer cells, and this dysregulation can be targeted for potential therapeutic benefit.

A5.7 MOVING AROUND: A NOVEL MECHANISM OF GLYCOGEN TRANSLOCATION IN ELASMOBRANCH ACID- AND BASE-SECRETING GILL CELLS

📅 MONDAY 3 JULY, 2017 ⌚ 14:40

👤 JINAE N ROA (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNITED STATES), MARTIN TRESGUERRES (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNITED STATES)

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In shark V-H⁺-ATPase (VHA)-rich base-secreting cells, VHA translocation to the cell membrane is regulated locally by the acid-base sensor soluble adenylyl cyclase (sAC). sAC is also present in Na⁺/K⁺-ATPase (NKA)-rich cells, where it likely regulates acid secretion. Both acid- and base-secreting cells are mitochondrion-rich (MR) cells; however, the energy source for those ATPases is unclear. We found that NKA- and VHA-rich MR cells were also enriched for glycogen and studied glycogen utilization in gills from starved and 24-hr post-fed sharks, as well as in isolated gill cells exposed to extracellular alkalosis. In acid-secreting cells, glycogen abundance was low in starved sharks and high in post-fed sharks, and the opposite was true for base-secreting cells. This indicated

differential energy use in those two cell types during net acid and net base secretion, respectively. Glycogen utilization in isolated gill cell experiments mirrored live shark results. In addition, we found that glycogen intracellular localization mirrored that of NKA and VHA. In acid-secreting cells, glycogen was present at the cell membrane together with NKA. In base-secreting cells, glycogen was present in the cytoplasm and translocated to the cell membrane together with VHA during acute (30 min) extracellular alkalosis. Furthermore, both VHA and glycogen translocation was blocked by the sAC-selective inhibitor KH7. These results highlight glycogen as an energy source in acid- and base-secreting cells, and reveal a novel mechanism to provide metabolic fuel to VHA involving rapid glycogen translocation within cells. Funded by APS Porter Fellowship to JNR and NSF IOS#1354181 to MT.

A5.8 THE PHYSIOLOGY OF THE TAMBAQUI (COLOSSOMA MACROPOMUM) AT PH 8.0

📅 MONDAY 3 JULY, 2017 ⌚ 14:55

👤 CHRIS M WOOD (UNIVERSITY OF BRITISH COLUMBIA, CANADA), RICK GONZALEZ (UNIVERSITY OF SAN DIEGO, UNITED STATES), ADALBERTO L VAL (INPA (AMAZON RESEARCH INSTITUTE), BRAZIL)

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The tambaqui is a model neotropical teleost which is of great economic and cultural importance in artisanal fisheries and commercial aquaculture. It thrives in ion-poor, often acidic Amazonian waters and exhibits excellent regulation of physiology down to water pH 4.0. Curiously however, it is reported to perform poorly in aquaculture at pH 8.0, an only slightly alkaline pH which would be benign for most freshwater fish. In initial experiments, we found that ammonia excretion was unchanged at pH's 4, 5, 6, and 7, but elevated after 24h at pH 8, exactly opposite the pattern seen in most teleosts. Subsequent experiments demonstrated that only ammonia, and not urea excretion was increased at pH 8.0, and that the elevation was proportional to a general increase in MO₂. There was an accompanying elevation in net acidic equivalent excretion and/or basic equivalent uptake which occurred mainly at the gills. Net Na⁺ balance was little affected while Cl⁻ balance became negative, implicating a disturbance of Cl⁻ vs base exchange rather than Na⁺ vs acid exchange. Arterial blood pH increased, reflecting combined metabolic and respiratory alkaloses. We conclude that a physiology designed to function well at acidic pH performs inappropriately at moderately alkaline pH. (FAPEAM, CNPq, Science without Borders, INCT-ADAPTA, NSERC Discovery).

A5.9 THE DEVELOPMENT AND PLASTICITY OF TWO KEY MECHANISMS IN ACID EXCRETION IN A MARINE TELEOST

📅 MONDAY 3 JULY, 2017 ⌚ 15:10

👤 JOSHUA K LONTHAIR (THE UNIVERSITY OF TEXAS AT AUSTIN, UNITED STATES), ANDREW J ESBAUGH (THE UNIVERSITY OF TEXAS AT AUSTIN, UNITED STATES)

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The prominent mechanisms for apical H^+ excretion following an acid-base disturbance are the Na^+/H^+ antiporters (NHEs) and the vacuolar-type H^+ ATPase (VHA); however, the degree to which each of these processes is utilised in marine teleosts is ambiguous. Similarly, there is little information about the development and plasticity of these systems in early life stage marine teleosts. This study attempts to discern the ontogeny and plasticity of the acid-base regulatory mechanism in red drum (*Sciaenops ocellatus*). Here, we present information on VHA, NHE2, and NHE3 at time points throughout development and after exposure to elevated pCO_2 . We found that VHA expression was significantly upregulated as early life development progressed, while no differences were found for NHE2 and NHE3. Significant up-regulation of NHE2 was observed in 36 hour post fertilisation larvae reared at 5000 μatm ; however, no effects were observed on NHE2 or VHA at any time point or pCO_2 . Confocal microscopy verified the presence of epithelial ionocytes at 36 hours post fertilisation, but found no significant reduction in ionocyte density as development progressed, although a decreasing trend was observed. Molecular support for NHE and VHA apical H^+ excretion will be phenotypically anchored using direct measurement via the scanning ion electrode technique and transporter specific inhibitors. Broadly, determining whether marine species exhibit phenotypic plasticity of apical H^+ excretion mechanisms following an acid-base disturbance can help predict the magnitude of the impacts of ocean acidification.

A5.15 ROLE OF PH REGULATION IN CORAL CALCIFICATION

📅 MONDAY 3 JULY, 2017 ⌚ 16:10

👤 SYLVIE TAMBUTTE (CENTRE SCIENTIFIQUE DE MONACO, MONACO)

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Precipitation of calcium carbonate depends on physico-chemical parameters, notably the concentration of carbonate (CO_3^{2-}) in the solution in which the reaction occurs. pH determines the equilibrium between dissolved inorganic species (DIC) including carbonate, and is thus considered to be a key parameter in calcification. In biological systems, measurements of pH at the sites of calcification are frequently included in mechanistic studies. In corals, the site of calcification, (referred to as the extracellular calcifying medium (ECM)), is not directly in contact with seawater but at the interface between tissues and skeleton. Until recently, most of the data on pH were not directly obtained from measurements in the ECM but derived from indirect approaches. These studies have shown that pH in the ECM is regulated above seawater pH and that this up-regulation is one of the parameters that explain the resistance/sensitivity of species to ocean acidification. Using work performed in my laboratory, I will illustrate how in vivo measurements on the first

stages of calcification have allowed us to obtain direct information on pH in the ECM, and also in the calcifying cells. I will show that under different environmental conditions, pH regulation in the ECM is a key parameter driving coral calcification, but that other parameters such as concentration of DIC and kinetics of reactions between DIC species are also important for the calcification process. Our molecular and physiological data suggest that calcium ATPases together with bicarbonate transporters and carbonic anhydrases are part of the toolkit used by corals to calcify.

A5.16 CORAL CELL PHYSIOLOGY: DISCOVERING NOVEL MECHANISMS IN THE LABORATORY AND TESTING THEIR RELEVANCE IN THE FIELD

📅 MONDAY 3 JULY, 2017 ⌚ 16:40

👤 MARTIN TRESGUERRES (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNITED STATES), MIKAYLA ORTEGA (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNITED STATES), ANGUS THIES (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNITED STATES), MEGAN E BARRON (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNITED STATES), DAVEY I KLINE (SCRIPPS INSTITUTION OF OCEANOGRAPHY - SMITHSONIAN TRI, UNITED STATES)

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Reef-building corals provide the structure for some of the most biodiverse ecosystems on earth. There is increasing concern about potential negative effects of environmental stress on coral; however, the lack of information about coral's essential physiological processes prevents understanding and predicting the extent of those effects based on mechanistic models. We have developed and/or validated antibodies against several coral proteins potentially involved in calcification and photosynthesis, including Na^+/K^+ -ATPase, $V-H^+$ -ATPase, Na^+/Ca^{2+} -exchangers, Rh-channels, Na^+/H^+ -exchangers, plasma membrane Ca^{2+} -ATPase, soluble adenylyl cyclase, and $slc4-HCO_3^-$ -transporters. Immunolocalization of laboratory-reared *Acropora yongei*, *Stylophora pistillata*, and *Pocillopora damicornis* revealed common and coral clade-specific cellular localizations of those proteins, supporting the existence of multiple mechanisms for ion transport for calcification and photosynthesis. To investigate the physiological roles of those proteins and their relevance in coral reefs, we conducted a combination of field and experimental aquarium experiments in Bocas del Toro (Panama) with wild *Orbicella franksi*, *Porites porites* and *Acropora cervicornis*. Preliminary results suggest that environmental conditions such as light, pH/ CO_2 , and organic nutrient levels can have a significant effect on the abundance and localization of ion-transporting proteins. These results reveal the presence of coral species-specific cellular mechanisms that might result in differential sensitivity to environmental stress, and highlight the importance of understanding basic coral cell physiology in multiple coral species and under diverse environmental conditions. Funded by NSF EF#1220641 to MT, and NSF OCE#1538495 to DIK and MT.

A5.17 BICARBONATE TRANSPORT REGULATES INTRACELLULAR pH CRITICAL FOR BIOMINERALIZATION IN THE SEA URCHIN LARVA

MONDAY 3 JULY, 2017

16:55

MARIAN Y HU (INSTITUTE OF PHYSIOLOGY UNIVERSITY OF KIEL, GERMANY)

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Efficient pH regulation is a fundamental requisite of all calcifying systems in animals and plants. Protons generated during biomineralization need to be buffered and excreted to promote further precipitation of CaCO_3 . The calcite endoskeleton of the sea urchin larva is formed through intracellular precipitation of amorphous CaCO_3 within vesicles of the primary mesenchyme cells (PMCs). To date, little is known about pH regulatory mechanisms of PMCs and their role in calcification. Here, we demonstrate the importance of a SLC4 family transporter in the acquisition of HCO_3^- , and intracellular pH (pH_i) regulation of PMCs. Among the four SLC4 family HCO_3^- transporter genes found in the genome of *Strongylocentrotus purpuratus*, SpSlc4a10 is predominantly expressed in PMCs and highest mRNA levels were detected during de novo formation of the larval skeleton. Morphants lacking this HCO_3^- transporter are characterized by decreased PMC pH_i and impaired ability to compensate an intracellular acidosis. These acid-base regulatory defects are accompanied by decreased calcification and spicule deformations. Pharmacological studies underline this observation and demonstrate DIDS sensitive pH_i regulation in PMCs. Reductions in seawater pH, resembling ocean acidification scenarios, led to an increase in SpSlc4a10 expression. This work identified and characterized a HCO_3^- transport mechanism critical for pH_i homeostasis of the calcifying PMCs in the sea urchin embryo. Since pH regulation is fundamentally linked to the biological precipitation of CaCO_3 , a better understanding regarding acid-base regulatory mechanisms in calcifying systems will be required to broaden our understanding regarding biomineralization strategies in animals.

A5.18 ELEVATED EXTRACELLULAR PH FACILITATES EARLY SHELL FORMATION UNDER OCEAN ACIDIFICATION IN MUSSEL LARVAE

MONDAY 3 JULY, 2017

17:10

KIRTI RAMESH (GEOMAR HELMHOLTZ CENTRE FOR OCEAN RESEARCH, GERMANY), MARIAN Y HU (INSTITUTE OF PHYSIOLOGY CHRISTIAN-ALBRECHTS-UNIVERSITY KIEL, GERMANY), JÖRN THOMSEN (GEOMAR HELMHOLTZ CENTRE FOR OCEAN RESEARCH, GERMANY), MARKUS BLEICH (INSTITUTE OF PHYSIOLOGY CHRISTIAN-ALBRECHTS-UNIVERSITY KIEL, GERMANY), FRANK MELZNER (GEOMAR HELMHOLTZ CENTRE FOR OCEAN RESEARCH, GERMANY)

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Marine calcifiers are amongst the most vulnerable organisms to environmental stress during their early life history stages. However, limited studies investigated the mechanisms underlying their early calcification processes and impaired performance under stress such as ocean acidification. Working with larval stages of the blue mussel, *Mytilus edulis*, we used miniature microsensors to study pH, carbonate and calcium concentrations at the site of mineralization, for the first time. Using 48 hour post fertilization D-veliger stages, we show that mussel larvae can increase pH and $[\text{CO}_3^{2-}]$ beneath the growing shell, leading to a ~1.5-fold elevation in calcium carbonate saturation state (Ω_{arag}) which translates into a 2.7 fold increase in carbonate precipitation rate. Larvae exposed to ocean acidification exhibit a continuous drop in pH and $[\text{CO}_3^{2-}]$ at the extracellular site of calcification, which correlates with decreased shell growth, and, eventually, shell dissolution at $\text{pCO}_2 > 2000 \mu\text{atm}$. Our findings help explain why calcifying bivalve larvae can form shells under moderate acidification scenarios and provide a direct link between ocean carbonate chemistry and larval calcification rate for the first time. The primary mechanisms of ion transport contributing to elevated pH and $[\text{CO}_3^{2-}]$ during calcification have been investigated in RNAseq analyses allowing us to develop a model of the ion regulatory network that facilitates biomineralisation in these organisms.

A5.19 EXTREME EXTRACELLULAR AMMONIUM ACCUMULATION IN TROPICAL DIAPAUSING COPEPODS: TRANSCRIPTOMIC AND METABOLOMIC INSIGHTS INTO TOLERANCE MECHANISMS

THURSDAY 6 JULY, 2017 09:00

FRANK MELZNER (GEOMAR, GERMANY), RAINER KIKO (GEOMAR, GERMANY), ANNA SCHUKAT (GEOMAR, GERMANY), FRANZ - JOSEF SARTORIS (GEOMAR, GERMANY), HOLGER AUER (UNIVERSITY OF BREMEN, GERMANY), LARS KRAEMER (ICMB KIEL, GERMANY), PHILIP ROSENSTIEL (ICMB KIEL, GERMANY), GEORG POHNERT (UNIVERSITY OF JENA, GERMANY)

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Surprisingly little is known about physiological processes underlying major life cycle transitions in key zooplankton species such as copepods. Larval stages of the tropical marine copepod (*Calanoides carinatus*) can descend into deeper, colder and hypoxic layers when environmental conditions are not optimal at the surface and enter diapause. During diapause, metabolic rates are reduced by 80-90%, thus enabling animals to survive long periods from endogenous reserves. Another adaptive mechanism aiding in energy conservation is the accumulation of extreme levels of ammonium in extracellular fluids: studying *C. carinatus* from the Namibian upwelling system, we detect ammonium >100mM, most likely in order to maintain neutral buoyancy. We used transcriptomic and metabolomic approaches to compare diapausing vs. non-diapausing copepods to learn more about ammonium tolerance mechanisms. We developed a novel instrument to fix copepods in 500m depth directly after capture with a plankton net to obtain high quality transcriptomes. While total and mRNA levels were greatly reduced during diapause, a suite of ca. 300 genes was strongly upregulated during diapause, among them genes involved in ammonium detoxification, the urea cycle and ammonia and ion transport. We also detected strong upregulation of hemerythrin-like sequences during diapause. Metabolomic analysis revealed that a key mechanism is the accumulation of high amounts of TMAO during diapause. However, contrary to expectations, concentrations of glutamate and glutamine were extremely low. In summary, we can provide a first comprehensive assessment of diapause physiology that sheds some light on how to survive ammonium concentrations non-vertebrate would tolerate.

A5.20 RELATIONSHIP BETWEEN OXIDATIVE STRESS, AMMONIA TOLERANCE AND BRAIN SWELLING IN THE GOLDFISH (*CARASSIUS AURATUS*)

THURSDAY 6 JULY, 2017 09:30

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The goldfish readily withstands prolonged anoxia and exposure to high environmental ammonia (HEA). In mammals such insults can lead to potentially fatal brain swelling. In mammals and fishes, the underlying mechanisms of brain swelling are unresolved, but oxidative stress is known to be involved in initiating post-anoxic- or ammonia-induced brain damage. To determine if brain swelling was related to oxidative stress, goldfish were acutely exposed to HEA (5 mmol L⁻¹ NH₄Cl) at two different acclimation temperatures (14°C, 4°C). At 14°C, exposure to HEA for 72 h resulted in a 20% increase in brain water volume, which was indicative of brain swelling. In addition, internal ammonia and glutamine concentrations were markedly elevated in the brain and liver, and there was evidence of oxidative damage in both tissues. However, the brain was more sensitive to oxidative stress, in which there was a 2-fold increase in thiobarbituric-acid reactive substances and a 3-fold increase in protein carbonylation. Greater activities of catalase, glutathione peroxidase, and glutathione reductase in the brain suggested that antioxidant capacity was upregulated to partially offset oxidative stress during hyperammonemia at 14°C. In contrast, acclimation to colder (4°C) water completely attenuated the oxidative stress response to HEA in both liver and brain, and there was no change in brain water volume, despite similar increases in internal ammonia and glutamine. These findings suggest that ammonia-induced oxidative stress initiates the swelling of goldfish brain during HEA, and that their high antioxidant capacity prevents additional damage.

A5.21 IS AMMONIA EXCRETION AFFECTED BY GILL VENTILATION IN RAINBOW TROUT *ONCORHYNCHUS MYKISS*?

THURSDAY 6 JULY, 2017 09:45

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Since ammonia is toxic to fish, excretion of ammonia through the gills occurs at about the same rate as its production, but the branchial transport mechanisms have long been controversial. Ammonia movement has been explained by simple diffusion of NH₃ along P_{NH3} gradients and/or by NH₄⁺ diffusion and/or Na⁺/NH₄⁺ exchange along electrochemical gradients, such that changes in ventilatory water flow would not affect ammonia efflux across the gills. However, elevated plasma ammonia directly stimulates fish ventilation,

which raises the question. Why do fish show ammonia-induced hyperventilation? The diffusion-limitation argument was made before the discovery of rhesus (Rh) glycoproteins which serve as ammonia excretion channels, greatly increasing gill ammonia permeability. Therefore, we hypothesized that ammonia-induced hyperventilation is used for facilitating ammonia excretion under circumstances of ammonia loading (e.g. feeding or exercise) when branchial ammonia permeability is increased by upregulated Rh proteins in fish gills. In order to test our hypothesis, we used variations in environmental O₂ levels to manipulate ventilation in trout studied under control or ammonia-loaded conditions? i.e. hyperventilation in moderate hypoxia or hypoventilation in moderate hyperoxia. Results indicate that under control conditions, ammonia excretion was insensitive to changes in ventilation, but after the fish were ammonia loaded by NH₄HCO₃ infusion for 30+h, increases in ventilation in ammonia-loaded fish elevated the ammonia excretion rate, while decreases in ventilation reduced the ammonia excretion rate, in accord with our hypothesis. Further research on the mechanisms of ammonia excretion, and the possibly involvement of Rh proteins, is required (NSERC Discovery).

A5.22 THE EFFECTS OF RHCGB KNOCKOUT ON NA⁺ UPTAKE BY LARVAL ZEBRAFISH (*DANIO RERIO*)

THURSDAY 6 JULY, 2017 10:00

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The mechanisms of Na⁺ uptake by larval zebrafish have been well-characterized, with many studies demonstrating an important role for Na⁺/H⁺-exchanger 3b (NHE3b). Integral to NHE3b function in low Na⁺, freshwater environments is the presence of the Rhesus glycoprotein Rhcg (formerly Rhcg1), an ammonia channel protein. In a simplified view, the efflux of ammonia down its partial pressure/electrochemical gradient is believed to facilitate the influx of Na⁺ against its concentration gradient. In previous work, morpholino knockdown of Rhcg led to a reduction in Na⁺ uptake by 4 days post-fertilization (dpf) zebrafish reared in low pH or low Na⁺; NHE3b function was likely thermodynamically limited in the absence of Rhcg facilitation. To elaborate on these findings, an *rhcg* knockout line of zebrafish was developed using the clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 nuclease system (1 base-pair insertion in exon 1). Wild-type and *rhcg* mutant larvae were reared in normal (pH 8; 800 μmol/l Na⁺), low pH (pH 4; 800 μmol/l Na⁺), and low Na⁺ (pH 8; 50 μmol/l Na⁺) conditions. Based on knockdown studies, we hypothesized that *rhcg* mutants would have lower Na⁺ uptake rates than wild-type larvae at 4 dpf under low pH and low Na⁺ conditions. However, *rhcg* mutant larvae had higher Na⁺ uptake rates than wild-types in normal conditions and had similar uptake rates to wild-types in low pH and low Na⁺. These preliminary results highlight the potential differences between knockdown and knockout approaches and may reflect compensatory changes in *rhcg* knockout not observed in response to knockdown.

A5.23 PROLACTIN IS A REGULATOR CONTROLLING ACID SECRETION FUNCTION IN ZEBRAFISH

THURSDAY 6 JULY, 2017 10:15

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Aquatic acidification caused by industrial pollution and climate change has threatened the survival of aquatic animals. To understand the primary link between environmental changes and physiological responses, the role of neuroendocrine system in acid-base regulation under pH disturbance in fish is an important issue. Zebrafish has been an emerging model for the researches on fish ion and acid-base regulation. A group of ionocytes with specific ion transporters was identified as the major cells responsible acid secretion function in zebrafish, and isotocin, cortisol, stanniocalcin, and estrogen-related receptor were demonstrated their actions on zebrafish body fluid acid-base homeostasis. Recently, we further identified prolactin (PRL) as another regulator involved in the control of acid secretion function in zebrafish. While only the expression of PRL receptor a (PRLRa) was stimulated by acidified water (pH 4) in adult gills, expressions of both PRLRa and -b were significantly increased in pH 4-treated zebrafish embryos. Loss of function experiments showed that knockdown of either receptor severely impaired acid secretion function in zebrafish embryonic skin where acid-secreting ionocytes are developed. Loss-of-function of either receptor decreased the cell density of acid-secreting ionocytes with concomitant suppression in the expression of the related ion transporters, suggesting that PRL participates in acid-base regulation through modulating the differentiation of ionocytes. The expression of FOXI3a and GCM2, 2 transcription factors control ionocytes differentiation, was differentially affected by knockdown of PRLRa and -b, implying the 2 receptors may mediate distinct pathways of ionocytes differentiation.

A5.24 COMPENSATION FOR OCEAN ACIDIFICATION RELEVANT CO₂ EXPOSURE CAUSES BROAD DOWNSTREAM CONSEQUENCES IN MARINE FISH

THURSDAY 6 JULY, 2017 10:55

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Marine fish are renowned for their ability to cope with ambient elevated CO₂ and readily survive exposures 5-25 times higher than what is predicted under near-future climate change scenarios. Decades of foundational research has revealed that nearly all fish recover internal pH homeostasis by elevating HCO₃⁻ during CO₂-induced acidosis. While effectively protecting pH, it is now believed that sustained elevation of pCO₂ and HCO₃⁻ may lead to a variety of secondary sub-lethal impairments. Under this unifying hypothesis, we set out to examine downstream consequences of CO₂ compensation in two different, but important physiological responses in marine fish: osmoregulation and neurosensory/behaviour. The gulf toadfish (*Opsanus beta*) was used in the first set of experiments to assess the impacts of 1900 μatm CO₂ on intestinal transport physiology using intact-animal and isolated tissue using chamber techniques. CO₂ exposure stimulated HCO₃⁻ loss through osmoregulatory pathways in the intestine that was counterproductive to whole-animal acid-base balance, incurring a tissue metabolic cost that would increase baseline metabolic demand. In the second experiment, avoidance behaviour and brain/plasma ion chemistry was measured during 1900 μatm CO₂ exposure in the spiny damselfish (*Acanthochromis polyacanthus*). Damselfish exposed to CO₂ exhibited significant behavioural disruptions and evidence of CO₂ compensation in the brain and the plasma. These results support the intriguing hypothesis that CO₂ compensation alters ion gradients across neuronal membranes, causing behavioral disruptions. Together, this work demonstrates broad downstream impairments following CO₂ exposure, suggesting CO₂ compensation protects pH but may come at physiological costs/tradeoffs that impact fitness in future oceans.

A5.25 RESPONSIVENESS OF ACID-BASE REGULATORS AND EPIGENETIC REGULATION IN TELEOST UNDER SEAWATER ACIDIFICATION

THURSDAY 6 JULY, 2017 11:10

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CO₂-induced seawater acidification has been recognized as an emerging abiotic stressor, potentially affecting ecosystems? Biodiversity, concordance and functions. Studies regarding the effects of acidification on marine organisms have been primarily conducted in several species; nevertheless, long-term transgenerational consequences regarding seawater acidification is still an unclear issue.

In this study, we applied marine India medaka (*Oryzias melastigma*) into CO₂-induced acidic challenges. Growth retardation in this marine species is not as significant as previous found in euryhaline Japanese medaka (*Oryzias latipes*). Moreover, transcripts levels of anion exchanger 1a (AE1a), Na⁺/HCO₃⁻ cotransporter a (NBCa) and Na⁺/H⁺-exchanger 2 (NHE2) were up-regulated in larvae as well as in adult gills/intestine under acidic perturbation. And the methylation level in AE1a promoter was significantly increased in parental ovary of CO₂-treated group. However, on one hand, methylation levels of those promoters in the CO₂-treated F1 offspring were not changed. On the other hands, transcripts levels of acid-base regulators and the H⁺ secretion ability were kept on up-regulated in CO₂-treated F1 offspring. Based on above results, we inferred that the parent may pass the genetic messages from the primary generation with epigenetic modifications; therefore, their progeny might be endowed with possible capacities to cope with CO₂-induced perturbations. In addition, AE1a, NBCa and NHE2 can effectively maintain intact homeostasis and be used as markers of epigenetic memory for teleosts to cope with seawater acidification.

A5.26 OSMOREGULATION AND ACID-BASE BALANCE IN TWO SPECIES OF MARINE CRABS IN RESPONSE TO ELEVATED CO₂ AND REDUCED SALINITY

THURSDAY 6 JULY, 2017 11:25

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Marine crustaceans are unusual amongst marine invertebrates by showing a variety of responses to salinity change; from independent regulation of body fluid osmolarity to conformity. Osmoregulators tend to be good iono regulators, and are also less vulnerable to elevated pCO₂ levels because of their capacity to compensate for acid-base disturbances (pH/PCO₂/HCO₃⁻) via mechanisms in the gills responsible for both acid-base and ion homeostasis. Little, however, is known about the combined effects of reduced salinity and CO₂-induced acidification on acid-base balance, although early studies indicate a dominance of ion regulation over acid-base adjustments. To further investigate this link, we examined acid-base responses of the estuarine crab, *Carcinus maenas*, and the subtidal edible crab, *Cancer pagurus*, exposed to different levels of pCO₂ (ambient/1,000 μatm) and salinity (S₂₅/S₃₃) for up to one year. In the osmoregulating species, *C. maenas*, haemolymph acid-base balance was unaffected by pCO₂ or salinity. Haemolymph osmolality remained unchanged in crabs held at S₃₃, but fell before recovering in crabs held at S₂₅. Na⁺/H⁺ ATPase (NKA) activity increased in the posterior gills at S₂₅, accompanied by an increase in gene expression for cytosolic and membrane bound carbonic anhydrase, and NKA. In the osmoconforming species, *C. pagurus*, acid-base balance was influenced by both pCO₂ and salinity. Haemolymph osmolality declined at S₂₅ and remained low, and branchial NKA activities and gene expression for both forms of carbonic anhydrase were unaffected. Differences in response, especially the reliance on ion exchange mechanisms, will be discussed in terms of survival prospects.

A5.27 ENERGETIC COST OF INTESTINAL ION TRANSPORT PATHWAYS IN MARINE TELEOSTS

THURSDAY 6 JULY, 2017 13:50

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A number of studies have attempted to determine the metabolic cost of osmoregulation in teleost fish by measuring standard or resting metabolic rates with the anticipation that metabolic demands would be lowest at isosmotic salinities. However, there is no consensus among these studies and estimates of osmoregulatory costs based on whole animal metabolic rates range from a 2-30% of resting metabolic rates. Both interspecies differences and difficulties in determining true standard metabolic rates not influenced by spontaneous activity/anxiety/stress contributes to the lack of consensus in field. Isolated osmoregulatory tissues have rarely been employed even though they avoid problems of organismal responses. The few studies using isolated tissues have focused on the gills and estimate cost in the range of 2-4%, a range that matches theoretical considerations. Such low metabolic demands are challenging to capture by measurements of intact animals. To date, no studies have attempted to quantify the metabolic demand of osmoregulatory processes by the marine teleost intestine despite its central role in osmoregulation. Multiple parallel Cl⁻ uptake pathways with different energetic efficiency are present in the marine teleost intestinal epithelium and suggests osmoregulatory metabolic demands comparable to that of the gill tissue. The least expensive Cl⁻ uptake pathway is via NKCC2 consuming 0.17 ATP/Cl⁻ while anion exchange via SLC26a6 is consuming ≥ 1 ATP/Cl⁻ yet, marine teleost fish employ the SLC26a6 pathway extensively. The theoretical intestinal contribution to marine teleost osmoregulation and the seemingly energetically expensive strategy of employing anion exchange will be discussed.

A5.28 METABOLIC COSTS OF OSMOREGULATION USING ISOLATED TISSUE RESPIROMETRY IN THE GULF TOADFISH (*OPSANUS BETA*)

THURSDAY 6 JULY, 2017 14:20

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Marine teleosts tolerate a wide range of salinities. At higher salinities, osmoregulatory control is maintained by the desalination of ingested seawater in the esophagus, solute-coupled water absorption in the intestine, and excretion of Na^+ and Cl^- at the gill. While the cellular mechanisms that drive osmoregulation in the gastrointestinal tract are well-characterized, their metabolic costs have been difficult to determine empirically. This is because whole-animal respirometry lack the resolution required to identify metabolic shifts in systems that ultimately only account for ~2.5% total body mass. However, the involvement of the Na^+/K^+ - and proton pumps, and their upregulation with salinity, suggests these osmoregulatory processes are energetically expensive. We therefore measured oxygen consumption in isolated tissues from Gulf toadfish (*Opsanus beta*) acclimated to 3 different salinities: 9ppt (isosmotic), 33ppt (seawater) and 60ppt (hypersaline). There was a significant increase in oxygen consumption ($1.14 \mu\text{mol g}^{-1} \text{h}^{-1}$) when anterior intestinal preparations of 33ppt-acclimated fish were compared with 9ppt-acclimated fish ($p < 0.01$). However, we found no evidence that seawater- or hypersalinity-acclimation increased metabolic demands in isolated esophageal preparations. Comparing metabolic demands associated with osmoregulation in the gastrointestinal tract and estimates of branchial osmoregulatory cost to whole animal metabolic rates reveal an overall metabolic cost of osmoregulation of 7-8% of standard metabolic rate. By determining metabolic costs associated with osmoregulation, this work is important to understand tradeoffs associated with salinity acclimation (eg. digestive efficiency), and model how other stressors (eg. ocean acidification and warming) can interact to undermine the acclimation response.

A5.29 OSMOREGULATORY ROLE OF THE GUT IN THE SEA LAMPREY (*PETROMYZON MARINUS*)

THURSDAY 6 JULY, 2017 14:35

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The present work assessed osmoregulatory differences for different intestinal regions (esophagus, anterior intestine, posterior intestine and rectum) among ammocoetes (larvae), transformants (metamorphosing stages 1 to 7) and full transformers sea lamprey (juveniles). In addition, freshwater (FW)-acclimated transformers specimens were acclimated for 3 weeks to seawater (SW, 35‰) and several osmoregulatory parameters were measured: NKA activity, drinking water rates, intestinal water and chlorides absorption, expression of key ion-transporting proteins [Na^+/K^+ -ATPase (NKA), $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter (NKCC) and cystic fibrosis transmembrane conductance regulator (CFTR)]. Intestinal NKA activity was higher in anterior intestine compared to posterior. This activity enhanced dramatically in metamorphosis stages 6 and later for anterior and posterior intestine. However, no differences in NKA activity during that time were observed in the esophagus. Plasma $[\text{Cl}^-]$ and muscle moisture (%) of FW- were lower than SW-acclimated juveniles at. Acclimation to SW significantly enhanced NKA activity in posterior intestine compared to FW juvenile. However, NKA mRNA expression levels showed a negative correlation with salinity. For NKCC and CFTR mRNA expression levels we found their expression significantly higher in rectum, but in any case showed correlation with salinity challenge. Drinking rates were ninefold higher in SW juveniles than in FW ammocoetes or juveniles. Moreover, the intestinal water absorption in anterior intestine was similar among FW and SW juveniles but in both groups anterior intestine had significantly higher intestinal water absorption rates compared to posterior. In conclusion our results provide new insights into the osmoregulatory role of the intestinal tract in sea lamprey through development and salinity challenges.

A5.30 DO CHANGES IN GUT MOTILITY REPRESENT AN OSMOREGULATORY STRATEGY FOR SALMONIDS MIGRATING TO SEA?

THURSDAY 6 JULY, 2017 14:50

JEROEN BRIJS (UNIVERSITY OF GOTHENBURG, SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), GRANT W HENNIG (UNIVERSITY OF VERMONT, UNITED STATES), ALBIN GRÄNS (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), ESMÉE DEKENS (UTRECHT UNIVERSITY OF APPLIED SCIENCES, NETHERLANDS), MICHAEL AXELSSON (UNIVERSITY OF GOTHENBURG, SWEDEN), CATHARINA OLSSON (UNIVERSITY OF GOTHENBURG, SWEDEN)

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When moving from freshwater to seawater, an immediate response of euryhaline teleosts is to start drinking the salty medium, as this is the only source of water in their new dehydrating environment. To maintain osmotic homeostasis, the imbibed seawater must be desalinated so that intestinal ion and water absorption can occur. Despite the potential benefits of increased mixing and transport of imbibed water for increasing the efficiency of absorptive processes, the effect of water salinity on intestinal motility in teleosts was unknown. By qualitatively and quantitatively describing *in vivo* intestinal motility of euryhaline rainbow trout (*Oncorhynchus mykiss*), this study demonstrates that in freshwater, the most common motility pattern consisted of clusters of rhythmic, posteriorly propagating contractions separated by periods of quiescence. This pattern closely resembled mammalian migrating motor complexes (MMCs). Following a transition to seawater, imbibed seawater significantly distended the intestine, which resulted in a two to three-fold increase in the frequency of MMCs, as well as a substantial increase in ripple-type contractions. These findings demonstrate that intestinal contractile activity of euryhaline teleosts is dramatically increased upon exposure to seawater, which aids in the maintenance of osmotic homeostasis as increased drinking and mechanical perturbation of fluids is necessary to optimize intestinal ion and water absorption. Finally, the temporal response of intestinal motility in rainbow trout transitioning from freshwater to seawater coincided with previously documented physiological modifications associated with osmoregulation and may provide further insight on the underlying reasons shaping the migration patterns of salmonids.

A5.31 CARDIOVASCULAR CONSEQUENCES OF OSMOREGULATION IN FISH

THURSDAY 6 JULY, 2017 15:05

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Seawater (SW) acclimation and adaptation in fish involves drinking and gastrointestinal absorption of ions and water, along with branchial and renal excretion of excess ions to maintain osmotic and ionic homeostasis. While such processes can be expected to involve cardiovascular changes, surprisingly little is known about this. Thus, in a series of experiments, we analysed the hemodynamic changes in rainbow trout (*Oncorhynchus mykiss*) during transition from freshwater (FW) to SW. FW acclimated trout exhibited an immediate drinking response when exposed to SW, while blood flow to the gastrointestinal tract gradually increased and doubled over a four day period. After SW acclimation (>6 wks.), gastrointestinal blood flow and cardiac output were elevated by approximately 120% and 35%, respectively. This was mediated by elevated stroke volume while heart rate was unchanged, and likely involved regional changes in vascular resistance. Further experiments showed that the elevated SV was associated with compact myocardial growth and increased cardiac filling pressure, suggesting active reductions in venous capacitance in SW. Trout in SW appeared to have maintained scope for increasing gastrointestinal blood flow after gavage feeding, but moderate warming (+6.5°C) compromised SV indicating reduced myocardial contractility and thermal tolerance of SW acclimated trout. Our data suggest that elevated cardiac output and gastrointestinal perfusion are fundamental and integrated responses of euryhaline fishes acclimating to SW. This likely serves convection of absorbed water and ions for excretion by osmoregulatory organs, as well as to supply oxygen and nutrients to the gut.

A5.32 5-HYDROXYTRYPTAMINE STIMULATES TRANSEPITHELIAL ION TRANSPORT ACROSS THE GASTRIC CAECUM OF MOSQUITO LARVAE: EFFECTS OF REARING IN FRESH WATER VERSUS BRACKISH WATER

THURSDAY 6 JULY, 2017 15:45

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Larvae of *Aedes aegypti*, the yellow fever vector, inhabit a variety of aquatic habitats ranging from fresh water to brackish water. Understanding larval physiology and how they adapt to different rearing salinities can provide the foundation for development of novel larvicides. This study focuses on the larval gastric caecum, an organ that has previously not been studied widely. We have shown that the regionalization of VA and NKA, and their ATPase activities, are dependent upon rearing salinity. We also provide the first measurements of H⁺, K⁺, and Na⁺ fluxes across the distal and proximal gastric caecum, and have shown that they differ in the two regions consistent with previously reported regionalization of ion transporters. These fluxes decrease over time, but were restored by 5-hydroxytryptamine (5-HT; 1 μM). Electrochemical gradients and transepithelial potentials for H⁺, Na⁺ and K⁺ are also altered by 5-HT. The initial transepithelial potential of +29 mV (lumen-positive) decayed to +12 mV within 6-8 mins and was restored to +40 mV by 5-HT. The lumen-positive TEP and lower pH of the caecal lumen contrasts the lumen-negative TEP and highly alkaline pH of the adjacent anterior midgut, indicating that the caecum is functionally distinct from the rest of the gut. Previous studies have shown stimulation of the anterior and posterior midgut ion transport of fresh water larvae by 5-HT. Our results show that 5-HT is important in the maintenance of ion transport across the gastric caecum in both fresh water and brackish water larvae.

A5.33 EXERCISE, TEMPERATURE, AND THE OSMORESPIRATORY COMPROMISE IN THE DOGFISH SHARK *SQUALUS ACANTHIAS SUCKLEYI*

THURSDAY 6 JULY, 2017 16:00

MARINA GIACOMIN (THE UNIVERSITY OF BRITISH COLUMBIA, CANADA), PATRICIA SCHULTE (THE UNIVERSITY OF BRITISH COLUMBIA, CANADA), CHRIS M WOOD (THE UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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When metabolic demand is elevated, fish upregulate gill ventilation and perfusion in order to increase oxygen supply. The characteristics of the gills that maximize gas exchange, such as large surface area and thin diffusion distance, may be the same as those that promote the diffusive exchange of solutes between blood and water. We investigated the potential physiological trade-offs at the gills of dogfish sharks after exhaustive exercise, which elevates metabolic oxygen demand. Oxygen consumption (MO₂), gill ventilation frequency and amplitude, in addition to ammonia, urea-N and

diffusive water fluxes (³H₂O exchange) at the gills were measured. While MO₂ almost doubled immediately after exercise, ventilation rate and amplitude exhibited only mild increases, suggesting that animals were increasing oxygen extraction efficiency. Ammonia and urea-N fluxes were elevated by 2.2 and 1.2 fold, and returned to pre-exercise levels after two hours in recovery, while diffusive water fluxes were unchanged after exercise. Surprisingly, recovery in hyperoxia (PO₂ = 310 Torr) did not seem to attenuate post-exercise hyperventilation, or the effects of the osmorepiratory compromise in terms of ammonia, urea-N, and diffusive water fluxes. These results are compared with responses of the dogfish shark to elevated temperature, another stimulant to metabolic demand. As temperature increased, diffusive water flux increased in parallel to oxygen consumption (MO₂), whereas ammonia and urea-N fluxes appeared to be independently regulated. Again, hyperoxia did not alleviate the osmorepiratory compromise (NSERC Discovery).

A5.34 ARE MEDITERRANEAN THREE-SPINED STICKLEBACKS (*GASTEROSTEUS ACULEATUS* L.) OF THE CAMARGUE WETLANDS WITH CONTRASTED SALINITY CONDITIONS MORPHOLOGICALLY AND PHYSIOLOGICALLY DIFFERENT?

THURSDAY 6 JULY, 2017 16:15

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Salinity acclimation capacities of southern three-spined sticklebacks (*Gasterosteus aculeatus* L.) living in different saline habitats of the Camargue area (Rhône delta, northern Mediterranean coast) were investigated. Individuals from lagoons with different salinity ranges and from freshwater canals were exposed to seawater (SW; 30 ‰), brackish water (BW; 15 ‰), or freshwater (FW; 5 ‰). Morpho-geometric measurements of sub-adult fish sampled from 1994 to 2017 were determined from fish inhabiting in these different habitats. Also, oxygen consumption rates and osmoregulatory parameters (branchial Na⁺/K⁺-ATPase, NKA activity and gene expression of the α1 subunit and α1a and α1b NKA isoforms, gill ionocytes morphology) were measured from fish living in three contrasted habitats and after exposure to different salinities.

At all the studied locations, only the leirismorphotype was observed with also limited morphological variations. No short-term effect of salinity could be detected on oxygen consumption from fresh, brackish, and salt water fish. In these animals, gill NKA activity was salinity-dependent with also less NKA α1b in FW than in SW-fish. Ionocytes in FW-fish gills were located along the lamellae and at their base, whereas these cells were restricted to gill filaments in SW-fish. Finally, electron microscopy revealed three different types of apical structures for these ionocytes: a honeycomb-like structure and a dome shape in FW, and deeply encrypted in SW.

Therefore, sticklebacks of the Camargue area living in contrasted saline conditions belong to a very homogenous euryhaline population and are not exposed to strong metabolic demands due to salinity changes.

A5.35 CHARACTERIZATION OF INTESTINAL OLEIC ACID UPTAKE STRATEGIES IN THE PACIFIC HAGFISH (*EPTATRETUS STOUTII*)

THURSDAY 6 JULY, 2017 16:30

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Hagfish are basal craniates that occupy a unique feeding niche. Previous enzymatic studies demonstrate preference for carbohydrate-fuelled energy, with lipids used secondarily to permit extended periods of food deprivation. Indeed, hagfish have distinctive intracellular lipid micelles within multiple tissues including archinephric duct, heart and intestine. Despite this unique morphological characteristic, few studies examine the nutritive uptake strategies of the hagfish, with no published accounts on lipid absorption. Thus, our aim was to characterize the mechanisms of intestinal lipid absorption in Pacific hagfish (*Eptatretus stoutii*). Intestinal flux studies were conducted with ^3H -oleic acid (OA) and demonstrate the presence of a saturable transporter ($K_m = 78.7$, $V_{max} = 143$) in the hindgut. We demonstrate significant post-prandial up-regulation of the OA transport and will examine expression and function using partial sequences of a putative fatty acid transport protein (FATP) isolated from a hagfish transcriptome. Mammalian models suggest insulin-dependent recruitment of specific FATP isoforms to apical membranes. To examine the role of insulin in hagfish OA transport, we conducted intestinal flux studies 24h following insulin injection. While metabolic oxygen consumption increased and demonstrates metabolic effects of insulin, there were no significant effects on OA transport. This is the first examination of lipid uptake in a basal member (craniate) of the vertebrate lineage and is a novel contribution to the understanding of lipid transport and the evolution of lipid acquisition strategies in vertebrates.

A5.36 EURYHALINE MUMMICHOGS EXPOSED TO SEAWATER AND HYPERHALINE CONDITIONS AUGMENT THE CATION-PERMEABLE PARACELLULAR PATHWAY BY DIFFERENTIALLY REGULATING CLAUDIN 10 ISOFORMS

THURSDAY 6 JULY, 2017 16:45

WILLIAM S MARSHALL (ST FRANCIS XAVIER UNIVERSITY,
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Salt-secreting epithelia secrete Na^+ passively down an electrochemical gradient via paracellular pathways uniquely localized between ionocytes and accessory cells (Sardet et al. J. Cell Bio. 80:96-117, 1979), but the protein composition of these cation-selective leaky junctions is unknown. We hypothesized that members of the claudin 10 family could be key junctional components, so euryhaline mummichogs (*Fundulus heteroclitus*) were transferred from freshwater (FW) to full-strength seawater (SW) and from SW to hypersaline conditions (2SW, 64 ‰) to measure transcriptional responses of claudin (*cldn*-)10 isoforms and *cftr* (cystic fibrosis transmembrane conductance regulator anion channel). Transfer from FW to SW increased *cftr* mRNA abundance 3-fold and *cldn-10d* and *-10e* 2-fold, compared to FW-FW controls, whilst *cldn-10c* and *-10f* mRNA abundance was unchanged. Transfer from SW to 2SW increased *cftr* mRNA abundance 3-fold and *cldn-10e* 2-fold but also *-10c* and *-10f* increased 4-fold. These transcriptional responses to 2SW coincided with increased number of leaky junctions (observed by TEM), compared with SW-SW controls. For both transfers, *cldn-10e* increased early (24h) and transiently, while other *cldn-10* isoforms increased later (3-7 days) and remained elevated. Changes in *cldn-10f* and *-10c* appear linked, consistent with the tandem repeat locus in the *Fundulus* genome, whilst mRNA encoding *cldn-10e* and *-10d*, also tandem repeats, seemed independently regulated. We conclude that differential regulation of *cldn-10* isoforms is critical to create and maintain cation-selective leaky junctions in teleost fish gills. Supported by NSERC (WSM, SPK, PMS), Skidmore Coll. (JPB) and NSF (CKT).

A5.10 FUNCTIONAL DEVELOPMENT OF PATHOGEN DEFENSE BY GASTRIC ALKALIZATION IN A BASAL DEUTEROSTOME

■ TUESDAY 4 JULY, 2017 POSTER SESSION

● JIA-JIUN YAN (INSTITUTE OF CELLULAR AND ORGANISMIC BIOLOGY (ICOB) ACADEMIA SINICA, TAIWAN), MEIKE STUMPP (GEOMAR HELMHOLTZ CENTER FOR OCEAN RESEARCH, GERMANY), MARKUS BLEICH (INSTITUTE OF PHYSIOLOGY CHRISTIAN-ALBRECHTS-UNIVERSITY KIEL, GERMANY), YI-HSIEN SU (INSTITUTE OF CELLULAR AND ORGANISMIC BIOLOGY (ICOB) ACADEMIA SINICA, TAIWAN), MARIA I. ARNONE (STAZIONE ZOOLOGICA ANTON DOHRN NAPOLI, ITALY), MARIAN Y. HU (INSTITUTE OF PHYSIOLOGY CHRISTIAN-ALBRECHTS-UNIVERSITY KIEL, GERMANY)

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Since over 100 years the sea urchin embryo has been used as a model organism for understanding the early developmental biology, gene regulatory network and cell specification of deuterostomes. In contrast to vertebrates that have highly acidic stomach, the stomach of sea urchin larvae is highly alkaline, reaching a pH of 9.5. Besides a role of food ingestion, the evolutionary advantage of an alkaline gut in the sea urchin larva remains unknown. Using vertebrate systems as a guide we hypothesize that alkaline digestive systems may also be the first step of innate pathogen defense in the sea urchin larva. Exposure to the marine pathogenic bacterium (*Vibrio diazotrophicus*) not only increased the number of immunocytes but also enhanced gastric alkalization significantly. Furthermore, the increased capacity for gastric pH regulation is accompanied by the up-regulation of a sodium hydrogen exchanger (*nhe2*), which is highly expressed in stomach epithelial cells. This transporter has been suggested to be involved in the export of protons from the stomach cells at the basolateral side. Our results offer the evidence that in convergence to vertebrates, the alkaline stomach of the sea urchin larva represents an important microbial filter for controlling the gut microbiome and gastrointestinal health.

A5.11 REGULATION OF BICARBONATE SECRETION IN MARINE FISH INTESTINE VIA THE CALCIUM SENSING RECEPTOR

■ TUESDAY 4 JULY, 2017 POSTER SESSION

● SÍLVIA F GREGÓRIO (CCMAR-CENTRE OF MARINE SCIENCE, PORTUGAL), JUAN FUENTES (CCMAR-CENTRE OF MARINE SCIENCE, PORTUGAL)

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In marine fish, high epithelial bicarbonate secretion by the intestine generates luminal carbonate precipitates of divalent cations that play a key role in water and ion homeostasis. Therefore, the present study was designed to expose the putative role for calcium and the calcium sensing receptor (CaSR) in the regulation of HCO₃⁻ secretion (BCS) in the intestine of the sea bream (*Sparus aurata* L.). Gastrointestinal fluids, blood plasma and intestinal tissue were collected after a single meal. Effects on the expression of the CaSR in the intestine were evaluated by qPCR and an increase in

expression was observed in the anterior intestine in fed fish in relation to unfed conditions. A parallelism to CaSR expression was observed in intestinal fluid calcium concentration. In light of these results, intestinal tissue was mounted in Ussing chambers to test the putative regulation of HCO₃⁻ secretion in vitro using the anterior intestine. BCS was sensitive to varying calcium levels in luminal saline and to calcimimetic compounds known to activate/block the CaSR i.e. NPS2143 and R-568. A regulatory role of calcium in intestinal HCO₃⁻ secretion associated with its availability in luminal fluids is proposed in the sea bream intestine. It appears, that this physiological effect is mediated by CaSR as shown by responsiveness to calcium levels and calcimimetic compounds. Calcium availability related either to salinity or feeding seems to play a key role in the regulation of intestinal ion/acid base physiology mediated by the CaSR.

Funding: FCT Portugal PTDC/MAR-BIO/3034/2014 & SFRH/BD/113363/2015 to SF.

A5.12 THE EFFECT OF FRESH WATER ION STRENGTH ON EXTRACELLULAR ACID-BASE REGULATION IN THE AIR-BREATHING PANGASIANODON HYPOPHthalmus

■ TUESDAY 4 JULY, 2017 POSTER SESSION

● CHARLES R HEWITT (UNIVERSITY OF AARHUS, DENMARK), MIKKEL T THOMSEN (AARHUS UNIVERSITY, DENMARK), CHRISTIAN DAMSGAARD (AARHUS UNIVERSITY, DENMARK), MARK BAYLEY (AARHUS UNIVERSITY, DENMARK)

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It has been suggested that air-breathing fish are poor regulators of extracellular pH (pH_e) during environmental hypercapnia. Two hypotheses have been forwarded to explain this trend, seen largely in Amazonian species. Firstly, that the general diminutive gill surface area typically seen in air-breathing fish, impairs their capacity for branchial ion exchange and secondly, that the rate of ion exchange for pH_e-regulation is limited by the low counter-ion concentration in some Amazonian tributaries. In contrast to this trend, the air-breathing striped catfish (*Pangasianodon hypophthalmus*), native to the Mekong river, is a capable pH_e regulator, able to elevate [HCO₃⁻] to >40 mM. Here, we test the effect of acclimation to low water ion strength on pH_e regulation. The Mekong river has 10 times the ion strength of the Amazonian tributaries and we hypothesised that the fish would be unable to effectively regulate at Amazonian ion levels. Fish were grown in either soft Mekong type water (110 μS/cm) or hard Danish water (660 μS/cm), catheterised and exposed to a PCO₂ of 22 mmHg for 72 h in Amazonian type low ion water (in μmol·l⁻¹: Na⁺: 15, Cl⁻: 16, K⁺: 9, Ca²⁺: 9, Mg²⁺: 2) during which PCO₂ and acid-base relevant ions were regularly sampled. In contrast to our hypothesis, both groups showed near complete pH_e regulation during the hypercapnic insult although the soft water acclimated group showed a more rapid response. These results will be discussed in terms of the two hypotheses, previous findings and the possible effects of acclimatisation on the animals.

A5.13 SOLUBLE ADENYLYL CYCLASE IN TROUT RED BLOOD CELLS: CLONING, CHARACTERIZATION, AND POTENTIAL PHYSIOLOGICAL ROLES IN CO₂/PH/HCO₃⁻ SENSING AND OXYGEN TRANSPORT

TUESDAY 4 JULY, 2017 POSTER SESSION

CRISTINA SALMERON (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNIVERSITY OF CALIFORNIA SAN DIEGO, UNITED STATES), ROB P ELLIS (UNIVERSITY OF EXETER, UNITED KINGDOM), DANIEL MONTGOMERY (UNIVERSITY OF EXETER, UNITED KINGDOM), ROD W WILSON (UNIVERSITY OF EXETER, UNITED KINGDOM), MARTIN TRESGUERRES (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNIVERSITY OF CALIFORNIA SAN DIEGO, UNITED STATES)

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Soluble adenylyl cyclase (sAC) is a CO₂/HCO₃⁻/pH sensing enzyme that produces the ubiquitous signalling molecule cAMP. Red blood cells (RBCs) experience extreme changes in CO₂/HCO₃⁻/pH that jointly regulate RBC's main physiological function: the transport of O₂ and CO₂ between the respiratory medium and tissues. In theory, sAC could regulate many of RBC's physiological mechanisms via PKA-dependent phosphorylation. However, sAC presence and function in vertebrate RBCs has not yet been characterized. Here, we report the cloning and biochemical characterization of the first sAC from a bony fish, the rainbow trout (*Oncorhynchus mykiss*) (rtsAC). Using RT-PCR, we cloned a partial 610 bp rtsAC mRNA sequence from RBCs. Western blotting using anti-rtsAC antibodies revealed a specific 110 kDa band, and immunostaining localized rtsAC predominantly in the cytoplasm. Next, we characterized cAMP producing activity in trout RBCs. Forskolin (10 mM), a potent agonist of transmembrane adenylyl cyclase (tmACs), activated RBC cAMP production ~100-fold, which was partially inhibited by the tmAC inhibitor, 2,5-dideoxyadenosine (DDA). However, incubation of RBCs in elevated [HCO₃⁻] did not affect cAMP production, which was also insensitive to the sAC inhibitor KH7. We are currently exploring the possibility that rtsAC in RBCs is activated by external CO₂ rather than HCO₃⁻, as well as potential roles of RBC rtsAC in regulating O₂ uptake and delivery during different acid/base conditions. Since sAC and cAMP are evolutionarily conserved, these potential mechanisms could be relevant for all vertebrates. Funded by a Company of Biologists travel grant JEBTF-161115 to CS and NSF-IOS#1354181 to MT.

A5.14 TEMPORAL CHANGES IN THE KIDNEY TRANSCRIPTOME OF PACIFIC SPINY DOGFISH FOLLOWING LOW SALINITY EXPOSURE

TUESDAY 4 JULY, 2017 POSTER SESSION

DYLAN M COLE (UNIVERSITY OF ALBERTA, CANADA), JENNIFER ROACH (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES), ANDREW WHITEHEAD (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES), GREG G GOSS (UNIVERSITY OF ALBERTA, CANADA)

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Salinity poses a significant challenge to many aquatic organisms, including fishes. Many elasmobranch species are unable to adapt to changes in salinity (stenohaline), while others are more physiologically equipped to deal with osmotic stresses (euryhaline). The extent of euryhalinity is species dependent and is spread across several elasmobranch taxa. Interestingly, the cellular and molecular mechanisms that permit euryhalinity in elasmobranchs have not been fully investigated. The Pacific spiny dogfish, *Squalus suckleyi*, is a partially euryhaline shark that largely resides in ocean waters but has been documented in brackish waters of ~21 ppt. Previous studies using dogfish have examined changes in plasma osmolytes, specific gene expression through qPCR, and enzyme activity through biochemical assays following low salinity exposure (21 ppt). However, no work has been done to capture the entirety of changes in gene expression following low salinity exposure. Here we have employed a comprehensive, RNA-seq approach using the Illumina HiSeq 4000 to examine the temporal changes in kidney gene expression following low salinity exposure (21 ppt). The kidney is a primary osmoregulatory organ involved in controlling plasma osmolytes (e.g. Na⁺, Cl⁻, and urea). Kidney tissue was sampled immediately following exposure for 0h, 12h, and 48h to allow for analysis of short and long term changes. This is the first study to examine differential gene expression in an elasmobranch.

A5.37 EFFECTS OF CHYTRID FUNGUS *BATRACHOCHYTRIUM DENDROBATIDIS* ON TIGHT JUNCTIONS IN AMPHIBIAN SKIN

■ TUESDAY 4 JULY, 2017 POSTER SESSION

● JULIA GAUBERG (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA), SCOTT P KELLY (YORK UNIVERSITY, CANADA), NICHOLAS WU (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA), REBECCA L CRAMP (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA), CRAIG E FRANKLIN (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA)

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Amphibians are recognised as the most threatened class of vertebrates on the planet, with nearly 30% of all species being globally threatened. A substantial causal factor in this decline is a cutaneous infection caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*). *Bd* disrupts the normal functioning of the skin and can lead to death of the infected animal. However, the mechanism behind how this occurs is still poorly understood. It has been suggested that *Bd* disrupts cutaneous osmotic and ionic regulation, and recently it has been demonstrated that *Bd* disrupts junctional components between amphibian skin cells. However, a key junctional component that controls the barrier properties of the skin, the tight junction (TJ) complex, has not been investigated. Several studies demonstrate that TJs are essential for proper skin function in amphibians and that they play a critical role in passive ion movement across this tissue. In other studies, using mammalian models, fungi and fungal toxins have been shown to disrupt TJs in the skin. But a link between fungal infection and the contribution of TJs to the loss of skin integrity in amphibians has yet to be examined. Despite this, there is enough information to hypothesise that *Bd* disrupts the molecular components of the TJ complex in the skin of amphibians in association with infection. This study is the first to characterize the TJ genes present in the skin of the green tree frog, *Litoria caerulea*, and examine the effect of *Bd* infection on select TJ genes in the skin.

A5.38 INTESTINAL RESPONSE TO HIGH CO₂ IN THE EUROPEAN SEABASS

■ TUESDAY 4 JULY, 2017 POSTER SESSION

● ALEXANDRA ALVES (CCMAR-CENTER OF MARINE SCIENCES, PORTUGAL), SILVIA SF GREGÓRIO (CCMAR-CENTER OF MARINE SCIENCES, PORTUGAL), RENATA C EGGER (CCMAR-CENTER OF MARINE SCIENCES, PORTUGAL), IGNACIO RUIZ-JARABO (CCMAR-CENTER OF MARINE SCIENCES, PORTUGAL), JUAN FUENTES (CCMAR-CENTER OF MARINE SCIENCES, PORTUGAL)

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Our current knowledge of marine fish in high pCO₂ environments is still scarce, including both physiological and endocrine responses. Therefore, here we investigated the impact of high pCO₂ (~1300 uatm) relevant to the future scenarios of ocean acidification in sea bass juveniles (*Dicentrarchus labrax*). The increase of pCO₂ was achieved by CO₂ injection and controlled daily, as other water quality parameters. After 5 weeks, blood and intestinal fluids

and tissue were sampled and analysed. Intestinal tissues were collected from individual fish and mounted in Ussing Chambers in *in vivo* like conditions, to measure bioelectrical parameters and bicarbonate secretion. Our results demonstrated that high pCO₂ in seawater affected plasma electrolytes and energetic substrates in plasma, with increased pH, osmolality, potassium and HCO₃⁻ concentration, paralleled by decreased phosphorus, total protein and glucose concentrations. High pCO₂ in seawater was without effect on the activity of ouabain-sensitive ATPase and Bafilomycin A1-sensitive ATPase activities in the intestine. However, in intestinal fluids, high pCO₂ in seawater resulted in a 2.5-fold increase in HCO₃⁻ and 4-fold increase in the production of carbonate aggregates in response to high CO₂. These results are in keeping with the 3-fold increase of bicarbonate secretion rates in response to high CO₂ *in vivo* as measured by pH-Stat *in vitro*. Taken together, our data exposes plasmatic effects of high pCO₂ and a consistent response in the epithelial handling of bicarbonate aggregate production in the intestine of sea bass in response to ocean acidification.

Funding: FCT Portugal PTDC/MAR-BIO/3034/2014 & SFRH/BD/113363/2015 to SF.

A5.39 SEAWATER ALKALINITY MODULATES THE RESPONSE OF THE SHORE CRAB *CARCINUS MAENAS* TO OCEAN ACIDIFICATION

■ TUESDAY 4 JULY, 2017 POSTER SESSION

● BASTIAN MAUS (ALFRED-WEGENER-INSTITUTE HELMHOLTZ CENTRE FOR POLAR AND MARINE RESEARCH, GERMANY), CHRISTIAN BOCK (ALFRED-WEGENER-INSTITUTE HELMHOLTZ CENTRE FOR POLAR AND MARINE RESEARCH, GERMANY), HANS O PÖRTNER (ALFRED-WEGENER-INSTITUTE HELMHOLTZ CENTRE FOR POLAR AND MARINE RESEARCH, GERMANY)

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This study investigated the metabolic responses and capacity for extra- and intracellular acid-base regulation in the shore crab *Carcinus maenas*, subjected to various settings of water physicochemistry for four weeks. Crabs were subjected to elevated water PCO₂, resembling near future ocean acidification, combined with reduced water alkalinity at normal and elevated PCO₂. Organismal functions like MO₂ and circulatory performance were measured using respirometry and *in vivo* MRI, respectively, using a newly developed setup for simultaneously monitoring cardiovascular activity, acid-base and energy status in experimental animals under future ocean conditions. Animal acid-base parameters and cellular energy status were investigated using ³¹P-NMR spectroscopy. Blood samples were analysed for ion concentrations, using ion chromatography. We found that *C. maenas* was able to maintain extra- and intracellular pH under all conditions, elevating haemolymph bicarbonate levels under hypercapnia. However, metabolic rates and blood flow were significantly depressed under hypercapnia at reduced water alkalinity, compared to hypercapnia at control alkalinity. These effects seemed to depend on water alkalinity, rather than PCO₂ or water pH, suggesting a role for ambient carbonate and bicarbonate levels in whole-animal responses. While the exact mechanisms have not been identified, the findings demonstrate a need for integrative approaches to investigations of ocean acidification impacts.

A5.40 LOCAL ADAPTATION OF THE COMMON SEA STAR *ASTERIAS RUBENS* TO DIFFERENT SALINITIES

TUESDAY 4 JULY, 2017 POSTER SESSION

● LARA SCHMITTMANN (GEOMAR HELMHOLTZ CENTRE FOR OCEAN RESEARCH KIEL, GERMANY), TRYSTAN SANDERS (GEOMAR HELMHOLTZ CENTRE FOR OCEAN RESEARCH KIEL, GERMANY), CHRISTIAN BOCK (ALFRED WEGENER INSTITUT HELMHOLTZ CENTRE FOR POLAR AND MARINE RESEARCH, GERMANY), FRANK MELZNER (GEOMAR HELMHOLTZ CENTRE FOR OCEAN RESEARCH KIEL, GERMANY)

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The brackish Baltic Sea is a challenging environment for osmoconforming species such as the common sea star *Asterias rubens* and mechanisms of adaptation to low salinities are unknown. Osmoconformers rely on compatible organic osmolytes (OO) to adjust their intracellular osmolality to that of the surrounding medium while keeping inorganic ion concentrations stable. The salinity at which OO are depleted has been proposed a 'critical salinity' (S_{crit}) at which fitness becomes zero. We hypothesized that S_{crit} is shifted to lower salinities in Baltic Sea *Asterias* populations. We conducted a common garden experiment with animals from the North Sea (32 PSU) and the Baltic Sea (16 PSU) to investigate the effect of changing salinities on OO concentration and composition. Growth, feeding rate and righting time were measured and 1H -NMR spectroscopy was used to quantify intracellular OO. Higher salinities increased growth and feeding rates significantly for both populations. Righting time was significantly higher in 16 PSU and for animals from the North Sea. Glycine was found to be the main osmolyte in *Asterias rubens* and contributes up to 90% to the total OO pool. Osmolytes that are differently utilized by the two populations are glycine, lysine, methylamine, taurine, threonine and valine. As the total OO concentration is higher for individuals from the North Sea, we suggest that sea stars from the Baltic Sea are not adapted to lower salinities via modification of the OO pool size but via modification of inorganic ion concentrations.

A5.41 THE TROPICAL CORALS' pH MICROENVIRONMENT EXAMINED UNDER CHANGING SEAWATER pCO_2 CONDITIONS

TUESDAY 4 JULY, 2017 POSTER SESSION

● MARLENE WALL (GEOMAR, GERMANY), GERTRAUD MARIA SCHMIDT (ALFRED-WEGENER INSTITUT, GERMANY), KATHARINA FABRICIUS (AUSTRALIAN INSTITUT OF MARINE SCIENCE, AUSTRALIA), DIRK DE BEER (MAX-PLANCK INSTITUTE FOR MARINE MICROBIOLOGY, GERMANY)

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We examined changes in the corals' internal (calcifying fluid between tissue and skeleton and within the tissue) and external (diffuse boundary layer) microenvironment using microsensors. *Galaxea* spp. corals cultured under present-day conditions were consecutively exposed to two elevated pCO_2 conditions (800 μatm and 1300 μatm), and instantaneous changes in their internal and external environment monitored. The pH within the diffusive boundary layer decreased immediately with increasing seawater pCO_2 . In contrast, the change in internal pH was not always observed. In cases where internal pH decreased, this change was delayed and often corals up-regulated the internal pH to remain similar to internal pH values measured under present-day pCO_2 . Even after 4 weeks of incubation at 3 different pCO_2 levels (present-day, 800 μatm and 1300 μatm), illuminated corals maintained a high internal pH irrespective of culturing conditions. In the dark, the internal pH was always slightly lower than the external seawater pH. Dark calcification decreased from 0.18 $\mu M cm^{-2} h^{-1}$ (22% of light calcification) at present-day conditions to zero under the highest pCO_2 condition. We conclude that the external pH together with the capacity for internal pH regulation as well as the strong differences in dark and light internal pH microenvironment will affect the corals' response to future elevated pCO_2 conditions. An improved understanding of differences in light and dark pH microenvironment as well as their effects on the holobionts' metabolic processes (photosynthesis, respiration, calcification) is critical to better predict the responses of tropical corals to a future high CO_2 world.

A5.42 CELLULAR MECHANISM FOR BIOMINERALIZATION IN THE OTHOLITH SAC EPITHELIUM OF CALIFORNIA YELLOWTAIL (*SERIOLA DORSALIS*)

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 GARFIELD T KWAN (SCRIPPS INSTITUTION OF OCEANOGRAPHY UNIVERSITY OF CALIFORNIA SAN DIEGO, UNITED STATES), TAYLOR R SMITH (UNIVERSITY OF CALIFORNIA SAN DIEGO, UNITED STATES), MARTIN TRESGUERRES (SCRIPPS INSTITUTION OF OCEANOGRAPHY UNIVERSITY OF CALIFORNIA SAN DIEGO, UNITED STATES)

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Otoliths are calcium carbonate structures biomineralized within endolymph-filled saccule of teleost fish, and mediate hearing and equilibrium functions. Recent studies found that exposure to future levels of elevated CO_2 /pH can result in significant increases in otolith size in some fish species. However, the cellular mechanisms for otolith biomineralization and their regulation remain poorly understood, and therefore the mechanistic basis for enlarged otoliths are unknown. In other epithelia such as gill, intestine, and kidney, mitochondrion-rich cells with Na^+/K^+ -ATPase (NKA), vacuolar H^+ -ATPase (VHA), and carbonic anhydrase (CA) play major roles in ion transport, and soluble adenylyl-cyclase (sAC) acts as an acid-base sensing enzyme that regulates their activity. In the present study, we investigated the presence of NKA, VHA, CA, and sAC in the otolith sac epithelium of California yellowtail (*Seriola dorsalis*) using western blotting and immunohistochemistry. This work contributes mechanistic understanding of otolith biomineralization, an essential step to understand why otoliths significantly enlarge when teleost fish are exposed to future CO_2 /pH levels. Future work will investigate potential changes in the abundance, activity, and intracellular localization in fish exposed to elevated CO_2 levels. GTK is funded by an NSF GRFP.

A5.43 IS THE OSMORESPIRATORY COMPROMISE LIMITING INVASIVE SPECIES?

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 PATRÍCIA G FERREIRA (CIIMAR - INTERDISCIPLINARY CENTRE OF MARINE AND ENVIRONMENTAL RESEARCH, PORTUGAL), HUGO FLÁVIO (CIIMAR - INTERDISCIPLINARY CENTRE OF MARINE AND ENVIRONMENTAL RESEARCH, PORTUGAL), HARRY HACKING (DTU-AQUA - NATIONAL INSTITUTE OF AQUATIC RESOURCES, DENMARK), JANET GENZ (UNIVERSITY OF WEST GEORGIA, UNITED STATES), JONATHAN MARK WILSON (CIIMAR - INTERDISCIPLINARY CENTRE OF MARINE AND ENVIRONMENTAL RESEARCH, PORTUGAL), JANE BEHRENS (DTU-AQUA - NATIONAL INSTITUTE OF AQUATIC RESOURCES, DENMARK), JON SVENDSEN (DTU-AQUA - NATIONAL INSTITUTE OF AQUATIC RESOURCES, DENMARK)

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The round goby (*Neogobius melanostomus*) is a benthic fish native to the brackish waters of the Black and Caspian Seas; however, it has invaded several brackish and freshwater areas in North America and northern Europe. Notably, there are no records of *N. melanostomus* in high salinity marine habitats and the physiological mechanisms potentially constraining the invasion into this environment are largely unknown.

The gills play major roles in gas exchange and ionic regulation and it has been hypothesized that an osmorepiratory compromise impacts performance of each process. The trade-off of the large gill exchange capacity ideal for gas exchange is greater passive ion fluxes. High ionic waters would result in greater passive ion uptake that would require greater active ion excretion. This osmoregulatory disturbance may interfere with fish invasion by disrupting the regular activity of the gills, thus modifying the usual physiological mechanisms.

To examine if the osmorepiratory compromise could constrain the invasion of *N. melanostomus* into high salinity environments, this study compared Na^+/K^+ ATPase activity of metabolic phenotypes exposed to 0, 15 and 30 ppt water. Additionally, we examined variation in two important MO_2 measures, standard metabolic rate (SMR) and maximum metabolic rate (MMR) when *N. melanostomus* is exposed to increasing water salinities. Fish with an initially higher MMR (at the control salinity - 0 ppt) are likely to be more challenged by environmental stressors than fish with a lower MMR. Our results will enable a better understanding of the physiological mechanisms that may constrain invasive species in the aquatic environment.

A6 THE OBLIGATION OF ACTIVITY - HOW DO ANIMALS GET FIT AND WHAT TAKES THEM OVER THE HILL?

ORGANISED BY: LEWIS HALSEY (UNIVERSITY OF ROEHAMPTON, UK)
AND SHAUN KILLEN (UNIVERSITY OF GLASGOW, UK)

A6.1 'FIT FOR PURPOSE' - RAISING THE QUESTION OF WHETHER AND HOW WILD ANIMALS MAINTAIN OPTIMAL PHYSICAL FITNESS

TUESDAY 4 JULY, 2017 10:30

LEWIS HALSEY (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM)

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I will raise and discuss the proposition that, similarly to 'Westernised' human populations, some animals undertake insufficient involuntary activity to 'keep fit' and therefore must supplement this with voluntary exercise; keeping fit may be vital for key behaviours such as escaping predators and out-competing conspecifics. In turn, the time and energy costs of such voluntary exercise could have important ecological implications for these species. At least some animals, however, appear to be able to 'get fit' without increasing activity levels; are such adaptations associated with life history and/or ecology? Animals that know when they need to be fit, e.g. for seasonal migrations, may be advantaged by increasing their physical fitness levels endogenously at the appropriate time; this would negate spending time and energy on extra physical activity to achieve the same ends. In contrast, some species that may be advantaged from greater physical fitness under certain unpredictable situations such as during periods when the landscape is particularly demanding to traverse or when predators have become more abundant. These animals may tailor their physical fitness to the particular needs of the moment, allowing their fitness to be shaped by the specific endogenous demands that their bodies experience.

A6.2 DO BAR-HEADED GEESE TRAIN FOR HIGH ALTITUDE FLIGHTS?

TUESDAY 4 JULY, 2017 11:10

LUCY A HAWKES (UNIVERSITY OF EXETER, UNITED KINGDOM), NYAMBAYAR BATBAYAR (WILDLIFE SCIENCE AND CONSERVATION CENTRE, MONGOLIA), PATRICK J BUTLER (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM), BEVERLEY CHUA (UNIVERSITY OF BRITISH COLUMBIA, CANADA), PETER B FRAPPELL (UNIVERSITY OF TASMANIA, AUSTRALIA), JESSICA U MEIR (NASA, UNITED STATES), WILLIAM K MILSOM (UNIVERSITY OF BRITISH COLUMBIA, CANADA), TSEVEENMYADAG NATSAGDORJ (WILDLIFE SCIENCE AND CONSERVATION CENTRE, MONGOLIA), NICOLE PARR (UNIVERSITY OF EXETER, UNITED KINGDOM), GRAHAM R SCOTT (MCMASTER UNIVERSITY, CANADA), JOHN Y TAKEKAWA (AUDUBON CALIFORNIA, UNITED STATES), MARTIN WIKELSKI (MAX PLANCK INSTITUTE FOR ORNITHOLOGY, GERMANY), MATTHEW J WITT (UNIVERSITY OF EXETER, UNITED STATES), CHARLES M BISHOP (BANGOR UNIVERSITY, UNITED KINGDOM)

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Exercise at high altitude is extremely challenging, largely due to hypobaric hypoxia (low oxygen levels brought about by low air pressure). Studies of humans at extreme high altitudes on Mount Everest have demonstrated precipitous declines in blood oxygen content above 8,400 metres, and consequently, very few humans have reached the summit of Mount Everest without supplemental oxygen and only do so after considerable acclimatisation and training. Bar-headed geese (*Anser indicus*) are renowned high altitude migrants, and although they appear to minimise altitude during migration where possible, they must fly over the Tibetan Plateau (mean altitude 4,500 metres) for much of their annual migration. This requires considerable cardiovascular effort, but no study has assessed the extent to which bar-headed geese may train prior to migration at high altitudes. Using implanted loggers that recorded heart rate, acceleration, pressure and temperature, we find no evidence of training for migration in bar-headed geese. Geese showed no significant change in summed activity per day or maximal activity per day. There was also no significant change in maximum heart rate per day or minimum resting heart rate, which may be evidence of an increase in cardiac stroke volume if all other variables were to remain the same. We discuss the strategies used by bar-headed geese in the context of training undertaken by human mountaineers when preparing for high altitude, noting the differences between their respective cardiovascular physiology.

A6.3 OUTRUNNING INFECTION: INTERACTIONS AND TRADE-OFFS BETWEEN IMMUNITY AND PHYSICAL PERFORMANCE

📅 TUESDAY 4 JULY, 2017 ⌚ 11:40

👤 SIMON BABAYAN (UNIVERSITY OF GLASGOW, UNITED KINGDOM)

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Throughout the animal kingdom, physical activity is essential for survival: from foraging to occasional explosive exercise during predator-prey interactions, getting fit and staying fit is essential. Benefits of physical activity can be more subtle too, as regular exercise is often encouraged for improving general health, including reducing susceptibility to seasonal infections. Indeed, moderate activity, by increasing the expression of cellular and humoral immunity and balancing hormone secretion, leads to lower incidence and severity of upper respiratory tract infections and better responses to immunisation. However, while acute strenuous exercise is reported to increase the expression of pro-inflammatory cytokines, which may help fight pathogens in the short term, it is also suspected of impairing cellular responses and thus resistance to infection. Conversely, the immune response to infection can affect physical fitness: infection induces sickness behaviour, leading to weight loss and low locomotor activity. Indeed, a sick individual usually gets eaten before the infection kills it. Taking a life history view can help disentangle these complex interactions: both skeletal muscles and the immune system require protein and energy for growth, maintenance, and responsiveness to external stressors, and both sometimes exert tissue damage requiring repair. Consequently, allocation trade-offs operate both within and between immune responses and physical activity, especially under limited resource availability. Thus, optimal immunological and physical performance will depend on the intensity, duration, and frequency of the physical activity and on the immunological challenges faced, within the constraints of available nutritional resources.

A6.4 ANIMALS, ACTIVITY AND IMMUNOLOGY

📅 TUESDAY 4 JULY, 2017 ⌚ 12:10

👤 KEVIN D MATSON (WAGENINGEN UNIVERSITY, NETHERLANDS)

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Human-health authorities frequently emphasize the importance of movement and exercise. For example, the U.S. Centers for Disease Control and Prevention proclaim on their website, "Only a few lifestyle choices have as large an impact on your health as physical activity". If one looks to nature, many wild animals appear, at least from a human perspective, to engage in exactly this type of active lifestyle. But where do animals actually fall along the spectrum from couch potato to elite athlete? And what are the consequences of activity in different species? Generally speaking, the impact of activity, and potentially voluntary exercise, on the health of animals is poorly understood, and one physiological system ripe for investigation is the immune system. The rapidly expanding field of exercise immunology, a branch of biomedical science focused on

humans, has already demonstrated a diversity of effects of exercise on immune function and disease resistance and susceptibility. These effects can range from beneficial to detrimental, depending on the circumstances. Using a similar framework to study animals that are moving, exercising, or otherwise physically exerting themselves might provide new insights into the relationships between animal movement and migration and disease resistance and susceptibility.

A6.5 EXERCISING AT THE EDGE: WHEN IS EXERCISE COSTLY?

📅 TUESDAY 4 JULY, 2017 ⌚ 13:40

👤 CARL SOULSBURY (UNIVERSITY OF LINCOLN, UNITED KINGDOM)

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Animals often perform energetically-costly behaviours as a part of sexual display. The amount and timing of these activities can be used by females as signals of male quality and can act as sexually-selected traits. The costs of these activities can be considerable and may play a crucial role in determining individual life history strategies. In particular, too much exercise may lead to subsequent costs and reduced survival. Balancing the benefits of high investment in exercise relative to the potential future costs is therefore critical. Using the lekking black grouse *Lyrurus tetrix* as an example, I show the primary determinants of when males start to exercise, what determines their peak investment and the factors that cause exercise to become too costly.

A6.6 THE EFFECT OF WATER DEPTH ON CANINE HEART RATE DURING UNDERWATER TREADMILL (UWTM) EXERCISE

📅 TUESDAY 4 JULY, 2017 ⌚ 14:20

👤 JAMES A SWANSON (HARTPURY COLLEGE UNIVERSITY CENTRE, UNITED KINGDOM), CATHRYN JORDAN (JOHN WILEY AND SONS, UNITED KINGDOM), ALISON P WILLS (HARTPURY COLLEGE UNIVERSITY CENTRE, UNITED KINGDOM)

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Hydrotherapy enhances injury rehabilitation in dogs. Determining the effect of water depth on HR (a measure of exercise intensity and suitability) during UWTM could help develop injury specific protocols. Eight dogs, acclimatised to UWTM exercise and HR monitoring, performed four, 2.5 minute, depth order randomised walks at four different depths, on two separate occasions. Dogs were acclimatised to room and water temperature before exercise. HR was recorded every 5 seconds using a Polar 810i monitor. Sessions were videoed to identify start/end points and validate readings. Treadmill speed remained constant. With increasing water depth: a.) HR increased, but not significantly ($X^2(3) = 3.179, P = 0.365$); b.) Mean HR showed significant (if minimal) absolute differences ($X^2(3) = 17.919, P = 0.000$), and HR plateaued; c.) Minimum HR significantly increased ($X^2(3) = 41.174, P = 0.000$), contributing to significant reductions in range ($X^2(3) = 17.209, P = 0.001$), and

inter-quartilerange ($X^2(3) = 13.176, P = 0.004$). This study supports previous research showing that with exercise, increased muscle Oxygen requirement is largely met through increased stroke volume (following the Frank-Starling law), not significant increases in HR. Also, that due to buoyancy effects, exercise remains sub-maximal and HR plateaus, with increasing water depth. It was difficult to control for animal excitability before and during trials, so further studies using a range of treatment scenarios are justified to build on these findings. Inclusion of HR variability analysis in these studies would give a more nuanced assessment of canine cardiac function under UWTM.

A6.7 NEW INSIGHTS INTO EXERCISE INDUCED CARDIAC REMODELING IN TROUT REVEALED BY PROTEOMIC ANALYSIS

TUESDAY 4 JULY, 2017 14:35

SARAH L ALDERMAN (UNIVERSITY OF GUELPH, CANADA),
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Rainbow trout hearts have a remarkable ability for molecular, structural, and functional plasticity, and the inherent athleticism of these fish makes them ideal models for studies in comparative exercise physiology. Indeed, several decades of research using exercise-trained trout has shown both conserved and unique aspects of cardiac plasticity induced by a sustained increase in the workload of the heart. Despite a strong appreciation for the outcome of exercise training, however, the temporal events that generate this phenotype are not known. In the current study we quantified the changes in the cardiac proteome of rainbow trout, *Oncorhynchus mykiss*, during the early phases (4, 7, and 14d) of a typical exercise-training regime to provide a comprehensive overview of the cellular changes responsible for developing a trained heart phenotype. Enhanced somatic growth was paralleled by cardiac growth to maintain relative ventricular mass. This was reflected in the cardiac proteome by the increased abundance of contractile proteins and cellular integrity proteins as early as Day 4, including a pronounced and sustained increase in blood vessel epicardial substance - an intercellular adhesion protein expressed in the vertebrate heart. An unexpected finding was that proteins involved in energy pathways, including glycolysis, β -oxidation, the TCA cycle, and the electron transport chain, were generally present at lower levels relative to Day 0 levels, suggesting a reduced investment in the maintenance of energy production pathways. However, as the fish demonstrated somatic and cardiac growth during the exercise-training program, this change did not appear to influence cardiac function.

A6.8 TRACKING FEMALES 24/7: INDIVIDUAL VARIATION IN FORAGING EFFORT DURING PARENTAL CARE AND RESPONSE TO EXPERIMENTALLY-MANIPULATED WORKLOAD

TUESDAY 4 JULY, 2017 14:50

TONY D WILLIAMS (SIMON FRASER UNIVERSITY, CANADA),
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Parental care is assumed to be costly, and life-history theory predicts that individuals that invest more in parental care should benefit in terms of number of the number or quality of offspring produced. However, the idea that parents that work "harder" produce more, fitter chicks is surprisingly poorly supported in the literature. One potential reason is that the commonly used metric of foraging effort, nest visit rate, doesn't provide a good measure of workload. We used an automated radio tracking system to measure total activity in female European starlings (*Sturnus vulgaris*) 24/7 during late incubation and chick-rearing, combined with video analysis of prey fed to chicks, and with wing-clipping to experimentally increase workload. Tracking data revealed unanticipated, novel, aspects of parental behaviour, e.g. a) putative nocturnal foraging bouts, but also, b) high repeatability of inter-individual variation in activity across breeding stages. Furthermore, analysis of total activity detected a significant response to experimental manipulation of workload that was not apparent when foraging effort was measured using nest visit rate. However, variation in overall activity was not correlated with metrics of behaviour reflecting parental effort (nest visit rate, total prey brought back to the nest - with the exception of a novel prey item taken in one year) or with the outcome of behaviour directed to parental care (brood size at fledging, etc). This suggests that "activity" measured 24/7 captures other behaviours that birds undertake independent of parental effort per se, but which might explain different 'strategies' for maximizing fitness.

A6.9 EXPLORING AGEING IN WILD VERTEBRATE POPULATIONS USING LONGITUDINAL FIELD DATA

📅 TUESDAY 4 JULY, 2017 ⌚ 16:00

👤 HANNAH FROY (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), SUE LEWIS (CENTRE FOR ECOLOGY AND HYDROLOGY EDINBURGH, UNITED KINGDOM), RICHARD A PHILLIPS (BRITISH ANTARCTIC SURVEY CAMBRIDGE, UNITED KINGDOM), JOSEPHINE M PEMBERTON (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), DANIEL H NUSSEY (UNIVERSITY OF EDINBURGH, UNITED KINGDOM)

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Despite early scepticism about the occurrence of senescence in wild animals, declines in survival and reproductive performance with advancing adult age are increasingly documented in natural populations. This is partly due to accumulating data from longitudinal field studies, which follow individuals throughout their lives, thus enabling the examination of ageing trajectories within individuals, and the testing of life-history theory predictions in an ecologically and evolutionarily relevant context. We use data from three such studies: wandering albatrosses breeding on Bird Island, South Georgia; red deer on the Isle of Rum; and Soay sheep on the St Kilda archipelago, Scotland. Demographic senescence is well documented in these systems, but the traits that underpin these declines are not well understood. In older animals, declines in various physiological traits may lead to age-related variation in composite traits like foraging performance, which will in turn have important consequences for fitness. However, characterising such behaviours is challenging, since they are highly variable both within and between individuals. We use high-resolution tracking data to compare foraging trips of wandering albatrosses across the age range, but fail to detect differences in various trip and activity parameters in later life. In contrast, the 30-40 years of census data from the ungulate populations is of lower temporal resolution but covers the entire lifespan of many individuals, and reveals age-related changes in home range area, habitat quality and location. This variation is associated with fitness components, suggesting that changes in space-use may contribute to demographic senescence in these systems.

A6.10 PHYLOGENETIC COMPARATIVE ANALYSIS OF THE RELATIONSHIP BETWEEN HAEMATOCRIT, LIFE-HISTORY VARIABLES AND ENERGY METABOLISM IN BIRDS

📅 TUESDAY 4 JULY, 2017 ⌚ 16:30

👤 JEFF KANG NIAN YAP (SIMON FRASER UNIVERSITY, CANADA), OLIVIA HSIN-I TSAI (SIMON FRASER UNIVERSITY, CANADA), TONY D WILLIAMS (SIMON FRASER UNIVERSITY, CANADA)

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Aerobic capacity is assumed to be one of the main predictors of endurance or the ability to sustain high workload in a wide range of animals and haematocrit (Hct) has been suggested as a key determinant of aerobic or metabolic performance through its role in oxygen transport and delivery. At the intraspecific level, numerous studies have reported increases in Hct (and/or haemoglobin levels) in response to experimentally increased flight costs or increased thermogenic demands. Furthermore, Hct varies markedly among individuals and through the annual cycle in free-living birds and it has been suggested that this reflects adaptive modulation of this trait to meet seasonal changes in energy demands, e.g. during migration or winter acclimatization. To our knowledge there has been no comprehensive, phylogenetically-controlled test of these ideas at the interspecific level. We compiled a comparative data set of Hct, various measures of metabolic rate, as well as ecological and life-history traits for 107 species of birds. We use this to rigorously test several hypotheses for adaptive variation in hematocrit in relation to a) migratory status, b) breeding range temperature, and c) altitude. Although Hct is clearly only one component of the complex machinery underpinning metabolic rate we then extend these general ideas to test relationships between Hct and basal metabolic rate, daily energy expenditure and activity metabolic rate, in the context of different models of energy management.

A6.11 FUNCTIONAL ANALYSIS OF BROWN ADIPOSE TISSUES OF A NON-HOMEOTHERMAL RODENT, NAKED MOLE-RAT

TUESDAY 4 JULY, 2017 POSTER SESSION

YUKI OIWA (HOKKAIDO UNIVERSITY, JAPAN), YUKO OKAMATSU (HOKKAIDO UNIVERSITY, JAPAN), MASAHIDE BONO (DBCLS, JAPAN), HIDEYUKI OKANO (KEIO UNIVERSITY, JAPAN), KAZUHIRO KIMURA (HOKKAIDO UNIVERSITY, JAPAN), KYOKO MIURA (HOKKAIDO UNIVERSITY, JAPAN)

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Naked mole-rat (NMR) is a poikilothermic animal that shows an extremely long lifespan (>30 years) and a low incidence of cancer. NMR has low basal metabolic rate (BMR) and low body temperature (about 32 degrees) at 30 degrees that is equivalent temperature in the wild underground colony. Although in general, low body temperature prevents higher activity and proceeding in underground need more energy than on ground, NMR can build a vast underground colony. Additionally, NMR can move actively at 30 degrees in our laboratory. To reveal the mechanisms that enable NMR to have active behaviour in spite of their low BMR and body temperature, we focused on their brown adipose tissues (BATs), an important organ for non-shivering thermogenesis (NST). Although NMR is reported to be unable to induce persistent NST in a cold environment, we found that NMR had a large amount of BATs. Furthermore, we found that NMR BATs had the beta-3 adrenergic stimulation-dependent thermogenic ability both in vitro and in vivo, in addition to higher Uncoupling protein-1 expression levels than white adipose tissues. We then monitored NMRs by thermal camera, and found that NMR BATs' thermogenesis occurred even when they housed at 30 degrees and individual NMR showed several levels of BATs' thermogenesis. Importantly, NMR BAT's temperature rose during exercise depending on beta-3 adrenergic stimulation. These findings give insights into the potential roles of NMR BATs to bridge the gap between low BMR and active behaviour of NMR.

A6.13 MOLECULAR MECHANISMS OF HYPOXIA TOLERANCE OF THE BRAIN OF DIVING MAMMALS

TUESDAY 4 JULY, 2017 POSTER SESSION

ANDREJ FABRIZIUS (INSTITUTE OF ZOOLOGY BIOCENTER GRINDEL, GERMANY), MARIANA LEIVAS MÜLLER HOFF (INSTITUTE OF ZOOLOGY BIOCENTER GRINDEL, GERMANY), LARS P FOLKOW (DEPARTMENT OF ARCTIC AND MARINE BIOLOGY UNIVERSITY OF TROMSØ, NORWAY), THORSTEN BURMESTER (INSTITUTE OF ZOOLOGY BIOCENTER GRINDEL, GERMANY)

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In most terrestrial mammals, the lack of oxygen results within a few minutes in an irreversible damage to the brain. The vulnerability of the brain is due to its high metabolic activity, and the difference between energy production and demand caused by hypoxia. The brains of diving mammals, such as whales and seals, may survive recurrent and extended periods of hypoxia caused by diving without damage. This cerebral tolerance is credited to a combination of behavioural, anatomical and physiological diving adaptations, such as bradycardia and constriction of peripheral blood vessels. However, little is known about the cellular and molecular mechanisms that contribute to the hypoxia tolerance of the brain. Electrophysiological studies demonstrated a remarkable tolerance of the brain neurons of the hooded seal (*Cystophora cristata*). To elucidate the possible molecular adaptations of the brain of the hooded seal, we investigated the brain transcriptome using RNA-sequencing (RNA-seq). The levels of 6,229 transcripts from the visual cortex of the hooded seal were compared with those from the visual cortex of the ferret, which is a terrestrial relative of the pinnipeds. In the seal brain, we found a general reduction of the expression levels of genes related to energy metabolism. Most importantly, we identified two potential candidate genes (S100B and clusterin), which have an unusually high expression level in the seal brain and thus may contribute to its unusual hypoxia tolerance.

A7 NATURALLY OCCURRING EXPERIMENTS: USING LIFE HISTORY EVENTS TO UNDERSTAND LOCOMOTOR PERFORMANCE

ORGANISED BY: NATALIE HOLT (UC IRVINE, UNITED STATES) AND ANGELA HORNER (UNIVERSITY SAN BERNADINO, UNITED STATES)

SESSION SPONSORED BY: SABLE SYSTEMS INTERNATIONAL AND THE COMPANY OF BIOLOGISTS

A7.1 PHYSICAL EFFECTS OF REPRODUCTION ON LOCOMOTION IN LIZARDS

📅 THURSDAY 6 JULY, 2017 ⌚ 09:00

👤 MARGUERITE A BUTLER (UNIVERSITY OF HAWAI'I AT MĀNOA, UNITED STATES), JULIO A RIVERA (UNIVERSITY OF HAWAI'I AT MĀNOA, UNITED STATES), JEFFREY A SCALES (CALIFORNIA STATE UNIVERSITY STANISLAUS, UNITED STATES), CHRISTINA N LINKEM (UNIVERSITY OF HAWAI'I AT MĀNOA, UNITED STATES), ELIZABETH R HENRY (UNIVERSITY OF HAWAI'I AT MĀNOA, UNITED STATES)

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One demand placed exclusively on the musculoskeletal system of females is maintaining locomotor performance with increasing load over the reproductive cycle. Since many terrestrial vertebrate taxa rely on running or jumping to escape from predators it is paradoxical that many of these same taxa can carry a large reproductive mass. Females may experience a number of physical effects, including increased mass, increased body volume (with compression of internal organs as they compete for space with eggs), and stretching of the axial musculature which may decrease locomotor performance. In addition, females immediately postreproduction experience a sudden loss of mass and volume. We have studied the changes in locomotor performance in *Iguana iguana* during reproduction and recovery, the effects of external loads on *Iguana iguana* and the small arboreal lizard *Anolis sagrei*, and simulated pregnancy via the use of implants in *Iguana iguana*. Overall lizards have some capacity for compensation in sprinting, but not jumping. During the run, lizards are able to decrease the swing phase of the stride until a point at which overall performance declines with increasing loads. The effects are more pronounced for reproductive loads as opposed to external loads, and we found effects of volume independent of mass in our implant experiments. Performance declines are much more pronounced immediately postreproduction, with observations suggesting a lack of stability and coordination and functional disruption of the axial musculature, indicating greatest vulnerability during the recovery from reproduction. The physiological effects of reproduction on locomotion are complex.

A7.2 DOES MUSCULOSKELETAL AGING DIFFER FROM DISUSE ATROPHY? MUSCLE CONTRACTILITY AND ISOFORM EXPRESSION IN EXTREMELY ATHLETIC AGED MICE

📅 THURSDAY 6 JULY, 2017 ⌚ 09:40

👤 ANGELA M HORNER (CAL STATE UNIVERSITY SAN BERNARDINO, UNITED STATES), ALEX BEECHKO (CAL STATE UNIVERSITY SAN BERNARDINO, UNITED STATES), BRYAN ROURKE (CAL STATE UNIVERSITY LONG BEACH, UNITED STATES), THEODORE GARLAND JR. (UNIVERSITY OF CALIFORNIA RIVERSIDE, UNITED STATES), MANNY AZIZI (UNIVERSITY OF CALIFORNIA IRVINE, UNITED STATES)

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Advanced aging is associated with a host of musculoskeletal pathologies, including declining muscle mass and force production, decreasing stiffness of elastic elements, and functional decline in neuromuscular control. These age-related declines may be conflated with disuse atrophy, however, which co-occurs with aging and produces grossly similar results. Thus, studies attempting to characterize age-related changes in muscle performance and isoform expression may attribute physiological changes to aging that are in part influenced by sedentarism. Exercise training like endurance running is known to mitigate musculoskeletal decline due to both aging and disuse, but most studies of exercise and aging are necessarily limited to cross-sectional analyses or short durations. Here we present data from a novel model for muscle aging, a line of mice selectively bred for over 80 generations for high levels of wheel running (HR mice). We divided mice from two lines (HR and Control) into cages with lifelong access to wheels or into standard rodent cages with no wheel access. We measured plantar flexor muscle contractility (peak isometric force, maximum shortening velocity, and peak power) from mice at six, nine, and 18 months of age, and analyzed muscle isoforms from mice at three and 18 months of age [AH1]. Muscle quality (force/muscle mass) is higher in HR mice broadly, and significantly higher in HR-W mice specifically. HR mice have a higher proportion of type I fibers, and similarly have slower maximal shortening velocities. Preliminary results suggest that fiber type shifts and accompanying changes in muscle contractility may be mitigated with endurance training.

A7.3 HINDLIMB MECHANICS AND RESPONSE OF JUMPING FROM COMPLIANT SUBSTRATES IN TREE FROGS

THURSDAY 6 JULY, 2017 09:55

CRYSTAL M REYNAGA (UNIVERSITY OF CALIFORNIA IRVINE, UNITED STATES), CAITRIN EATON (UNIVERSITY OF CALIFORNIA IRVINE, UNITED STATES), EMANUEL AZIZI (UNIVERSITY OF CALIFORNIA IRVINE, UNITED STATES)

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Arboreal frogs navigate complex environments and substrates with varying mechanical properties. These changes in substrate compliance and elasticity can impose challenges when jumping off structures like leaves or small branches. An optimal well-coordinated jump might allow for the recovery of elastic energy stored in the substrate and potentially amplify mechanical power by effectively adding an in-series spring to the hindlimbs. However, in a poorly coordinated jump the energy applied to the substrate is not recovered. We aim to understand whether jumping organisms modulate their hindlimb kinematics to maximize energy recovery from a compliant substrate. We have designed a software-controlled jumping platform equipped with a real-time feedback controller that allows us to modulate the compliance of the substrate. We quantify the kinetics and kinematics of Cuban treefrogs jumping-off platforms with varying mechanical properties. We find that these animals do recover a portion of the energy stored in the substrate, and do so by modulating limb stiffness and rate of extension when faced with a compliant substrate. These results highlight a potential trade-off between jumping performance (e.g. power, acceleration) and responsiveness in animals that rely on elastic mechanisms to amplify power. This work will serve to broaden our understanding of how animals sense and respond to complex environments with varied mechanical properties.

A7.4 PRACTISE MAKES PERFECT: OPTIMISATION OF LOCOMOTOR PERFORMANCE IN 'ARBOREAL' PARKOUR ATHLETES ILLUMINATES THE EVOLUTIONARY ECOLOGY OF GREAT APE ANATOMY

THURSDAY 6 JULY, 2017 10:10

LEWIS G HALSEY (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM), SUSANNAH K THORPE (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM)

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An animal's size is central to its ecology, yet remarkably little is known about the selective pressures that drive this trait. A particularly compelling example is how ancestral apes evolved large body mass in such a physically and energetically challenging environment as the forest canopy, where weight-bearing branches and lianas are flexible, irregular and discontinuous and the majority of preferred foods are situated on the most flexible branches at the

periphery of tree crowns. To date the issue has been intractable due to a lack of relevant fossil material, the limited capacity of the fossil record to reconstruct an animal's behavioural-ecology and it not being possible to measure energy consumption in freely moving apes. We studied the oxygen consumption of parkour athletes while traversing an arboreal-like course as an elite model ape to test the ecomorphological and behavioural mechanisms by which a large-bodied ape could optimize their energetic performance during tree-based locomotion. Our results show that familiarity with the arboreal-like course allowed the athletes to substantially reduce their energy expenditure. Furthermore, athletes with larger arm-spans and shorter legs were particularly adept at finding energetic savings. Our results flesh out the scanty fossil record to offer evidence that long, strong arms, broad chests and a strong axial system, combined with the frequent use of uniform branch-to-branch arboreal pathways, were critical to off-setting the mechanical and energetic demands of large mass in ancestral apes.

A7.5 ONTOGENETIC DETERMINANTS OF ESCAPE PERFORMANCE IN EASTERN COTTONTAIL RABBITS (*SYLVILAGUS FLORIDANUS*)

THURSDAY 6 JULY, 2017 10:55

JESSE W YOUNG (NORTHEAST OHIO MEDICAL UNIVERSITY, UNITED STATES), ADAM D FOSTER (CAMPBELL UNIVERSITY, UNITED STATES), GREGORY A SMITH (KENT STATE UNIVERSITY, UNITED STATES), GABRIELLE A RUSSO (STONY BROOK UNIVERSITY, UNITED STATES), MICHAEL T BUTCHER (YOUNGSTOWN STATE UNIVERSITY, UNITED STATES)

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Juvenile animals are often under greater predation risk than adults. Here, we present an integrated dataset on musculoskeletal growth, locomotor performance (i.e., acceleration), and survivorship in an ontogenetic sample of 61 Eastern cottontail rabbits (*Sylvilagus floridanus*; mass: 106-1434g; estimated age: 22-208d). Cottontails are independent at an early age and experience high predation during their first year, making this an ideal model in which to investigate how selection may act on juvenile performance. We found that accelerations peaked in juvenile animals (i.e., masses: 850-950g; ages: 110-122d) and tapered at lower and higher body sizes, such that ontogenetic changes in acceleration were better explained by a quadratic than linear models (likelihood ratio tests: $p < 0.001$). Acceleration was strongly associated with the net mechanical work performed on the center of mass ($r^2 = 0.85$), and more specifically with work production at the lumbar spine, hip, and ankle joints. Though hindlimb extensor muscle mass and cross-sectional areas at these joints generally scaled with mechanical similarity during growth (i.e., positive allometry), effective mechanical advantage at the hip and ankle was significantly greater in younger rabbits ($p < 0.032$). Increased muscle leverage would permit younger animals to generate greater propulsion for a given amount of input force, suggesting a morphological underpinning for increased locomotor performance. Nevertheless, mortality data for a subset of animals fitted with radio-collars showed that peak acceleration was not significantly associated with survivorship to reproductive age ($p \geq 0.096$), indicating a complex relationship between locomotor performance and reproductive fitness. Supported by NSF IOS 1146916, 1146851 and 1147044.

A7.6 EFFECT OF HEAD SHAPE CHANGE WITH GROWTH ON THE DOLPHIN DRAFTING OF BOTTLENOSE DOLPHIN

📅 THURSDAY 6 JULY, 2017 ⌚ 11:25

👤 MAAKO MIYAKE (TOKAI UNIVERSITY, JAPAN), YUKINA MARUSHIMA (TOKAI UNIVERSITY, JAPAN), YOSHINOBU INADA (TOKAI UNIVERSITY, JAPAN), FUMIO TERASAWA (ENOSHIMA AQUARIUM, JAPAN), MAI SAKAI (KINDAI UNIVERSITY, JAPAN), TADAMICHI MORISAKA (MIE UNIVERSITY, JAPAN)

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In nature, mother dolphin helps its calf swim together side by side using a hydrodynamic effect called venturi effect. This behaviour is called “dolphin drafting”. In our previous study, we conducted wind tunnel tests using streamline models based on the shape of dolphins to investigate this behaviour and clarified that the dolphin drafting was beneficial not only for the calf but also for the mother. In this research, we focused on the effect of head shape change with growth on the dolphin drafting of bottlenose dolphin. A newborn calf has a short rostrum and its relative length increases as the calf grows. We conducted wind tunnel tests using models with different rostrum length and found that the short rostrum model, or the streamline model, had larger drag reduction than the long rostrum model showing that the newborn calf with a short rostrum can get larger benefit from the dolphin drafting than the elder one with a long rostrum. In consequence, the dolphin drafting could appropriately benefit the calf in accordance with the growth.

A7.7 MECHANISMS MEDIATING NATURALLY OCCURRING VARIATION IN INSECT FLIGHT PERFORMANCE

📅 THURSDAY 6 JULY, 2017 ⌚ 13:50

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Studies of animal locomotor performance do not often examine natural variation in this trait. Yet for many species, it is variation in locomotor performance that determines who succeeds under the naturally selective processes that drive individual fitness outcomes (e.g., the ability to secure a mate and produce offspring). This may be particularly true for animals whose fecundity crucially depends on their ability to interact with their environment while in flight. Ongoing work in my laboratory examines biotic- and abiotic factors that affect physiological mechanisms that mediate variation in male dragonfly flight- and flight muscle performance throughout life history. The naturally occurring experiment that has driven much of this work is an intestinal protozoan infection that in dragonflies results in a disease syndrome akin to mammalian diabetes and obesity. Susceptibility of adult dragonflies to this infection is highly variable in nature, and we have found it to be dependent on the chemical composition of the environment dragonflies encounter. In this talk I will integrate previously published and new data derived from this system to show how dragonfly larval life history appears to ultimately determine adult locomotor performance outcomes in the context of this host-parasite interaction. In addition, I will discuss how this project has recently driven the development of a new research direction in my lab focused on mechanisms controlling interactions between thermal and locomotor performance in flying insects.

A7.8 LOCOMOTOR PREFERENCES IN TERRESTRIAL VERTEBRATES: AN ONLINE CROWDSOURCING APPROACH TO DATA COLLECTION

📅 THURSDAY 6 JULY, 2017 ⌚ 14:30

👤 JOHN J LEES (LINKÖPING UNIVERSITY, SWEDEN), JAMES GARDINER (UNIVERSITY OF SALFORD, UNITED KINGDOM), JAMES USHERWOOD (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), ROBERT NUDDS (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

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Understanding how animals move within their environment is a burgeoning field of research. Despite this, relatively basic data, such as the locomotor speeds that animals choose to walk at in the wild, are sparse. If animals choose to walk with dynamic similarity, they will move at equal dimensionless speeds, represented by Froude number (Fr). Fr may be interpreted from simple limb kinematics obtained from video data. Here, using Internet videos, limb kinematics were measured in 112 bird and mammal species weighing between 0.61 and 5400 kg. This novel method of data collection enabled the determination of kinematics for animals walking at their self-selected speeds without the need for exhaustive fieldwork. At larger sizes, both birds and mammals prefer to walk at

slower relative speeds and relative stride frequencies, as preferred F_r decreased in larger species, indicating that F_r may not be a good predictor of preferred locomotor speeds. This may result from the observation that the minimum cost of transport is approached at lower F_r in larger species. Birds walk with higher duty factors, lower stride frequencies and longer stance times compared to mammals at self-selected speeds. The trend towards lower preferred F_r is also apparent in extinct vertebrate species.

A7.9 BIOMECHANICS OF INSECT INJURY REPAIR

📅 THURSDAY 6 JULY, 2017 ⌚ 14:45

👤 MAEVE O'NEILL (TRINITY COLLEGE DUBLIN, IRELAND),
DIEGO DELANDRO (TRINITY COLLEGE DUBLIN, IRELAND),
DAVID TAYLOR (TRINITY COLLEGE DUBLIN, IRELAND)

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We investigated the ability of desert locusts (*Schistocerca gregaria*) to repair injuries and restore mechanical strength to their limbs. This was done for insects of a number of different ages to evaluate if there was a downward trend for repair ability with increasing age, as commonly seen in mammals. Whilst younger insects were somewhat better at restoring mechanical strength than their older counterparts (one week old versus 13/14 weeks old) the difference was minimal. Younger insects could restore damaged limbs to 82% of their initial strength (failure strength of 143.24 MPa versus 164.26 MPa for uninjured cuticle), and older insects to 73% (136.45 MPa versus 178 MPa for uninjured cuticle) still sufficiently strong for normal locomotion. Targeted deposition was observed in all cases. Investigations into different wound types were carried out to evaluate the effect of cuticular layer displacement on repair, and whether or not insects are optimally designed to resist certain types of injuries. It was discovered that insects are better able to resist puncture wounds compared to scalpel wounds. Additionally it was found that older insects are more susceptible to crack growth and thus failure from an injury than their younger counterparts. This is believed to be due to the ever increasing stiffness of the older insects cuticle, which leaves them vulnerable to crack growth.

A7.10 ADAPTIVE AIRSPEED ADJUSTMENT AND COMPENSATION FOR WIND DRIFT IN THE COMMON SWIFT: DIFFERENCES BETWEEN DAY AND NIGHT

📅 THURSDAY 6 JULY, 2017 ⌚ 15:00

👤 ANDERS HEDENSTRÖM (LUND UNIVERSITY, SWEDEN),
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Migratory birds are known to be capable of adjusting their heading direction to compensate for wind drift and adjust airspeed adaptively with respect to head- and tail winds. The shape of the power-speed relationship, which in turn is derived on the basis of flight mechanics, dictates appropriate airspeed adjustment. High flying nocturnally migrating common swifts have been shown to compensate for wind drift, but they failed to adjust airspeed as expected (increase in headwind and decrease in tail wind in relation to neutral wind). We present new measurements of diurnally migrating common swifts at a coastal site in the Baltic, where the birds did adjust airspeed adaptively during spring and autumn migration. During autumn migration they compensated for lateral wind drift by adjusting heading direction similarly to high altitude migrants in autumn. We also recorded flight speed and wind compensation during a summer weather related exodus, when the birds behaved similarly to those during autumn migration, although they showed a small degree of wind drift. The reason for why birds failed to adjust airspeed adaptively at high altitude is discussed, and we argue there is a threshold in the sensory system to detect small changes in optic flow based on visual landmarks.

A7.11 MOBILITY DURING MOULTING IN CRUSTACEA

📅 THURSDAY 6 JULY, 2017 ⌚ 15:45

👤 JENNIFER R A TAYLOR (SCRIPPS INSTITUTION OF OCEANOGRAPHY UC SAN DIEGO, UNITED STATES)

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The punctuated growth (moulting) process of crustaceans presents unique biomechanical challenges that can significantly disrupt locomotion and behaviour repeatedly throughout ontogeny. When the old exoskeleton is shed and a new one is formed during each moult cycle, its mechanical properties change so dramatically that it is temporarily unable to resist the forces of muscle contraction necessary for movement and locomotion. Remarkably, animals such as crabs, shrimps, and lobsters remain mobile during this precarious time by switching to a hydrostatic skeletal support mechanism. Locomotor performance, however, is significantly reduced for all modes of crustacean locomotion, including swimming, walking, and climbing. While the gaits for each of these locomotor modes remain unaffected by moulting, the kinematics changes significantly, especially for animals with larger body mass and those that live in the terrestrial environment, without the buoyant support of water. Thus, smaller and younger aquatic crustaceans are more robust to the mechanical challenges of moulting, which aids in predator avoidance during these vulnerable periods when escape locomotion is their primary defence. For larger and terrestrial crustaceans, locomotion may be hindered by moulting, with significant impacts on their behaviour and survival. Exploring the biomechanics of locomotion and moulting is therefore essential to understanding the life histories and diverse ecologies of crustaceans, especially in their transition from aquatic to terrestrial environments.

A7.12 AN AGEING MODEL TO EXPLORE THE ROLE OF CONTRACTILE AND CONNECTIVE TISSUE INTERACTIONS IN SKELETAL MUSCLE PERFORMANCE

📅 THURSDAY 6 JULY, 2017 ⌚ 16:15

👤 NATALIE C HOLT (NORTHERN ARIZONA UNIVERSITY, UNITED STATES), EMANUEL AZIZI (UC IRVINE, UNITED STATES)

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Skeletal muscle performance depends on the complex interactions between the force generating contractile tissues, and the force transmitting connective tissues. However, the spatially complex and functionally interdependent nature of this interaction makes it difficult to determine the various roles of these tissues, therefore limiting our understanding of muscle function. Here we propose that the phenotypic changes associated with ageing provides a natural perturbation to explore the interaction between contractile and connective tissues. Ageing is accompanied by significant changes to the mechanical properties of connective tissues structures. Hence, by determining the effect of age on muscle performance, we can make inferences about the fundamental role of connective tissue. Here we present data from multiple studies using a rat model of ageing. We show that: 1) whole muscle shortening, and therefore work, is decreased with age; 2) that whilst whole muscle shortening is decreased fiber bundle shortening is not; and 3) that the way in which the fibers in pennate muscle reorient during contraction varies with age. Taken together, these findings suggest that connective tissues modulate muscle shape change in a way that can affect muscle performance, and that changes to connective tissue structures have greater effect on performance at the muscle level than at the cellular level. These types of studies not only advance our basic understanding of muscle physiology, but also provide insights into the causes of, and variability in, the decline in muscle performance with age.

A7.13 PARENTAL AND ENVIRONMENTAL DETERMINANTS OF SWIM PERFORMANCE IN LARVAL ANEMONEFISH

WEDNESDAY 5 JULY, 2017 POSTER SESSION

• DAPHNE CORTESE (CRIOBE USR 3278 EPHE, FRANCE), ZOE SCHOLTZ (CRIOBE USR 3278 EPHE, FRANCE), SUZANNE C MILLS (CRIOBE USR 3278 EPHE, FRANCE), TOMMY NORIN (UNIVERSITY OF GLASGOW, UNITED KINGDOM), SHAUN KILLEN (UNIVERSITY OF GLASGOW, UNITED KINGDOM), RICARDO BELDADE (CRIOBE USR 3278 EPHE, FRANCE)

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According to Darwinian evolution, favourable and heritable traits that increase fitness in a given environment should be selected for. While ultimate fitness is difficult to quantify in relatively long-lived animals, the immediate effects on performance may provide a first glimpse at the process of evolution. Certain morphological traits in fish associated to swimming performance have been shown to vary according to local environmental cues. Whether these traits are heritable has not yet been shown. Here, we test the hypothesis that in a coral reef fish morphological traits correlated to swimming performance (such as total length and pectoral fin size) are heritable. Furthermore we test whether the same morphological traits are correlated with larval swimming performance. Finally, we test whether parental and larval traits are environment-dependent. Using offspring from breeding orange-fin anemonefish (*Amphiprion chrysopterus*) we show that larval total length and pectoral fin size regress linearly with the same maternal traits. Secondly, we show that these traits are associated to larval critical swimming speed. Finally we show that larvae from different breeding pairs have significantly different swimming performances and that these correlate with water currents. From the moment of hatching until settlement (the dispersal stage), larvae with better swimming performance should also be able to better disperse and find a suitable settlement habitat. If such differences in traits and swimming performance are kept throughout the whole dispersal stage (until 17 days post-hatching) then the maternal origin of larvae may translate into higher larval survival and may be a powerful evolutionary force.

A8 CONSTRAINTS ON ADAPTATION AND PERFORMANCE: FROM INDIVIDUALS TO POPULATIONS

ORGANISED BY: SHAUN KILLEN (UNIVERSITY OF GLASGOW, UK)
AND CAROL BUCKING (YORK UNIVERSITY, CANADA)

A8.1 PLASTICITY OF LOCOMOTOR FUNCTION AND ITS EFFECT ON BEHAVIOUR

📅 MONDAY 3 JULY, 2017 ⌚ 09:00

👤 FRANK SEEBACHER (UNIVERSITY OF SYDNEY, AUSTRALIA)

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The capacity to move is one of the most fundamental aspects in animal biology, and it influences biodiversity, fitness, and behaviour. Many animals have an astounding capacity to function well in a broad range of environments, while others are vulnerable to relatively small changes. These differences are intriguing considering that the principal molecular and physiological components are similar in most animals. Locomotor performance is of particular interest, because it is at the interface between physiology and behaviour. Any environmental parameter that limits locomotor performance and its underlying physiology will therefore also constrain behaviour. I will present recent work aimed at understanding the regulation and plasticity of locomotor performance in response to interactions between environmental factors, and their downstream effects on behaviour.

A8.2 PLASTICITY, PERFORMANCE, AND PACE OF LIFE: INDIVIDUAL DIFFERENCES IN PHYSIOLOGICAL AND BEHAVIOURAL FLEXIBILITY IN RESPONSE TO DAILY CHANGES IN TEMPERATURE AND OXYGEN AVAILABILITY

📅 MONDAY 3 JULY, 2017 ⌚ 09:40

👤 TOMMY NORIN (UNIVERSITY OF GLASGOW, UNITED KINGDOM), NEIL B. METCALFE (UNIVERSITY OF GLASGOW, UNITED KINGDOM)

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Individual animals differ consistently in both their physiology and behaviour; this variation sets the “pace of life” of individuals under different environmental conditions. Given the increased frequency of extreme weather events already occurring as a result of climate variability, the ability to adjust (or maintain) performance when faced with rapid environmental changes is likely to affect individual performance and fitness. Here, we show that individual European minnow (*Phoxinus phoxinus*) vary in both their metabolic and behavioural flexibility when exposed to daily changes in temperature and oxygen availability (hypoxia). Individual fish with relatively high standard (resting) metabolic rates (SMR) and activity levels were found to be more responsive to increased temperatures compared to their slower-paced conspecifics, but no differential plasticity was observed in their maximum metabolic rate (MMR). Contrarily, exposure to hypoxia constrained the MMR (but not SMR) of high-metabolic-rate fish relatively more than low-metabolic-rate individuals, whereas all fish exhibited a similar reduction in activity and related behaviours under hypoxia. Despite significant intraspecific variation in both physiological and behavioural traits we found limited evidence for co-variation between metabolic rate and behaviour. Finally, consistency of metabolic and behavioural traits was context dependent, such that repeatability of most traits was higher at higher temperatures and oxygen levels. Together, these results show that the ability of particular phenotypes to respond to rapid environmental changes depends on the environmental parameter, and that the strength of any selection for specific phenotypes likely will depend on the environmental stressors the animals are faced with.

A8.3 PARASITES AND HOST PERFORMANCE: INCORPORATING INFECTION INTO OUR UNDERSTANDING OF ANIMAL MOVEMENT

MONDAY 3 JULY, 2017 09:55

SANDRA A BINNING (UNIVERSITY OF NEUCHÂTEL, SWITZERLAND), DOMINIQUE G ROCHE (UNIVERSITY OF NEUCHÂTEL, SWITZERLAND), ALLISON K SHAW (UNIVERSITY OF MINNESOTA, UNITED STATES)

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Studies of animal locomotion and movement largely assume that individuals are healthy and performing at the best of their abilities in ways which are adapted to their survival. However, wild animals face numerous ecological challenges that can compromise their health, reduce their performance capacity, impair their movement abilities and, ultimately, lower their fitness. By diverting resources and increasing host energetic demands, parasites, bacteria and viruses (hereafter parasites) can dramatically influence the ways in which their hosts allocate energy to movement. Yet, the role of parasites in influencing patterns of animal locomotor performance and movement remains relatively unexplored, perhaps because animals often hide outward signs of sickness, and parasites tend to be small and inconspicuous to researchers. In this talk, I will review how parasite infection can lead to increased or decreased host locomotor performance and movement via impacts on host morphology and physiology. I will also give examples of behavioural strategies that some hosts employ to help overcome the disadvantages imposed by infection. Finally, I will highlight some recent theoretical and empirical research investigating the important role that pathogens and parasites play in driving the evolution of seasonal migration and large-scale host movement patterns more broadly.

A8.4 AEROBIC SWIMMING REVEALS A SUB-LETHAL THRESHOLD FOR TOLERANCE OF ACUTE WARMING IN FISHES

MONDAY 3 JULY, 2017 10:25

FELIPE R BLASCO (UFSCAR, BRAZIL), ANDREW J ESBAUGH (UTAU, UNITED STATES), SHAUN S KILLEN (GLASGOW, UNITED KINGDOM), EDWIN W TAYLOR (BIRMINGHAM, UNITED KINGDOM), F TADEU RANTIN (UFSCAR, BRAZIL), DAVID J MCKENZIE (CNRS MONTPELLIER, FRANCE)

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In fishes, tolerance of acute warming is typically measured using the critical thermal maximum (CT_{max}) protocol, where the animal is heated at a certain rate (e.g. $1^{\circ}C$ every 30 min) until loss of equilibrium (LOE). Although this test can be performed quickly on many animals, its weaknesses are (among others) that (1) in the wild, LOE would be an incipient lethal threshold whereas ecologically relevant effects may occur at less severe levels of warming, and (2) the reason for LOE is not known so the value of the protocol for understanding mechanisms of heat tolerance is limited. We exposed two species, Nile tilapia *Oreochromis niloticus* and pacu *Piaractus*

mesopotamicus, to a CT_{max} protocol while they were swimming at 85% of their maximum aerobic speed. Individuals of both species stopped swimming at a temperature approximately $2^{\circ}C$ below their own CT_{max} as measured under standard static conditions. Cessation of swimming was preceded by a switch from a steady aerobic swimming gait to an anaerobic burst-and-coast gait. Fatigue from aerobic exercise represents a less incipiently lethal threshold than LOE and one with a mechanism may be easier to reveal. Respirometry was performed, to investigate whether the fatigue threshold is linked to constraints on the ability of the fishes to meet the oxygen demands of sustained aerobic exercise plus an inexorable increase in standard metabolic rate with warming.

A8.5 CONSTRAINTS ON RAISING YOUNG IMPOSED BY PHYSIOLOGY IN GOLDEN HAMSTERS

MONDAY 3 JULY, 2017 10:40

SARAH A OHRNBERGER (UNIVERSITY OF VETERINARY MEDICINE VIENNA, AUSTRIA), CATHERINE HAMBLY (UNIVERSITY OF ABERDEEN, UNITED KINGDOM), JOHN R SPEAKMAN (UNIVERSITY OF ABERDEEN, UNITED KINGDOM), TERESA G VALENCAK (UNIVERSITY OF VETERINARY MEDICINE VIENNA, AUSTRIA)

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Traditional ecology taught us that mammals might be constrained by food availability in their habitat. Modern laboratory research and ad libitum feeding conditions during lactation however reveal that lactating females in fact might be limited by the dissipation of excessively produced metabolic heat. If endogenous heat production exacerbates when females raise young, have peak energy intakes and produce milk, any possible alleviation of the heat load might affect the given physiological limitation. Clearly, lactation is the most energy demanding process known in females when they reach 6-8 times resting metabolic rate. By breeding golden hamsters at three different ambient temperatures ($5^{\circ}C$, $22^{\circ}C$, $30^{\circ}C$) and assessing their lactation performance we sought to shed light on their physiological limitation during reproduction. We observed that body temperatures in lactating females were $0.5^{\circ}C$ higher than in non-reproductive controls ($F_{1,123}=13.6$, $p < 0.01$) with only small differences between the different ambient temperatures. Interestingly, milk energy production and energy intake was highest at 5° and significantly different from $22^{\circ}C$ and $30^{\circ}C$. Female golden hamsters have the shortest gestation time among eutheria but yet have very large litters of 3-16 pups. We conclude that females at $10^{\circ}C$ showing metabolic rates of over 10 times resting levels were less constrained than individuals at higher temperatures and we speculate that these relationships clearly affect free-living rodent populations as well.

A8.6 EVOLUTION, PLASTICITY, AND THE INTEGRATIVE PHYSIOLOGY OF PERFORMANCE IN HIGH-ALTITUDE ENVIRONMENTS

📅 MONDAY 3 JULY, 2017 ⌚ 14:00

👤 GRAHAM R SCOTT (MCMASTER UNIVERSITY, CANADA)

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High-altitude environments provide fertile ground for investigating the mechanisms and evolution of the physiological systems underlying animal performance. The cold and oxygen-depleted ('hypoxic') environment at high altitudes requires that endothermic animals sustain high rates of O_2 consumption for thermogenesis and locomotion while facing a diminished O_2 supply. My research examines the ways in which high-altitude natives overcome these constraints, in an effort to understand the evolution and plasticity of complex physiological systems. I will present our work on the respiratory, cardiovascular, and mitochondrial mechanisms of high-altitude adaptation in deer mice (*Peromyscus maniculatus*). High-altitude populations have an enhanced respiratory capacity (VO_2 max) in hypoxia compared to their low-altitude counterparts, due to evolved differences and environmentally-induced plasticity. This adaptive increase in VO_2 max arises from functional enhancements across the O_2 transport pathway, including lung O_2 uptake, O_2 circulation in the blood, and tissue O_2 extraction. The basis for these evolved differences in systems-level function involve coordinated changes at tissue, cellular, and transcriptomic levels of organization. Therefore, high-altitude adaptation involves a series of integrated changes across the O_2 pathway, and allows highland deer mice to overcome the strong environmental constraints on performance at high altitudes. Supported by NSERC of Canada.

A8.7 CARDIOVASCULAR CONTROL AND HIGH-ALTITUDE ADAPTATION IN DEER MICE (*PEROMYSCUS MANICULATUS*)

📅 MONDAY 3 JULY, 2017 ⌚ 14:40

👤 OLIVER H WEARING (MCMASTER UNIVERSITY, CANADA),
KEVIN B TATE (TRUMAN STATE UNIVERSITY, UNITED STATES),
GRAHAM R SCOTT (MCMASTER UNIVERSITY, CANADA)

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Endotherms must cope with the combination of cold and hypoxia to survive in high-altitude environments. High-altitude natives somehow maintain the high metabolic rates needed for aerobic thermogenesis while avoiding the maladaptive effects of chronic hypoxia. Here, we investigated whether differences in autonomic control of the cardiovascular system contribute to the increased thermogenic capacity (VO_2 max) of deer mice native to high altitudes. We compared captive breeding colonies derived from wild populations at high and low altitudes, acclimated to normoxia or hypobaric hypoxia (12 kPa O_2), and measured VO_2 max following intraperitoneal injection of adrenergic agonists/antagonists. Following control saline injection, highland mice had higher VO_2 max than lowland mice. However, these differences could not be explained by differences in α_1 -adrenergic tone on the heart, or β_2 - or α -adrenergic tone on the vasculature. However, the effects of the

α -adrenergic antagonist phentolamine was reduced in lowlanders after hypoxia acclimation, suggesting that a reduction in α -mediated vasoconstriction in thermogenic tissues might help increase VO_2 max in lowlanders after hypoxia acclimation. Nevertheless, our results suggest that population differences in VO_2 max do not arise from evolved changes in autonomic control of the cardiovascular system. Ongoing work is using implantable telemetry in freely-behaving animals to examine whether differences in cardiovascular function might instead contribute to population differences in the hypoxia acclimation response under routine conditions. Supported by NSERC of Canada.

A8.8 INADEQUATE FOOD INTAKE AT HIGH TEMPERATURES IS RELATED TO DEPRESSED MITOCHONDRIAL RESPIRATORY CAPACITY

📅 MONDAY 3 JULY, 2017 ⌚ 14:55

👤 KARINE SALIN (UNIVERSITY OF GLASGOW, UNITED KINGDOM),
SONYA K AUER (UNIVERSITY OF GLASGOW, UNITED KINGDOM),
GRAEME J ANDERSON (UNIVERSITY OF GLASGOW, UNITED
KINGDOM), COLIN SELMAN (UNIVERSITY OF GLASGOW, UNITED
KINGDOM), NEIL B METCALFE (UNIVERSITY OF GLASGOW,
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Animals, especially ectotherms, are highly sensitive to the temperature of their surrounding environment. Extremely high temperature, for example, induces a decline of average performance of conspecifics within a population, but individual heterogeneity in the ability to cope with elevating temperatures has rarely been studied. In this study, we examined inter-individual variation in feeding ability and consequent growth rate of juvenile brown trout *Salmo trutta* acclimated to a high temperature (19°C), and investigated the relationship between these metrics of whole-animal performances and among-individual variation in mitochondrial respiration capacity. Food was provided *ad libitum* yet intake varied ten-fold amongst individuals, resulting in some fish losing weight whilst others continued to grow. Almost half of the variation in food intake was related to variability in mitochondrial capacity: low intake (and hence growth failure) was associated with high leak respiration rates within liver and muscle mitochondria, and a lower coupling of muscle mitochondria. These observations, combined with the inability of fish with low food consumption to increase their intake despite *ad libitum* food levels, suggest a possible insufficient capacity of the mitochondria for maintaining ATP homeostasis. Individual variation in thermal performance is likely to confer variation in the upper limit of an organism's thermal niche and in turn affect the structure of wild populations in warming environments.

A8.9 THE EFFECT OF ELEVATED CO₂ ON SWIMMING PERFORMANCE AND SCHOOLING IN A CORAL REEF FISH SPECIES

📅 MONDAY 3 JULY, 2017

🕒 15:10

👤 LAUREN E NADLER (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNITED STATES), AMY COX (JAMES COOK UNIVERSITY, AUSTRALIA), PAOLO DOMENICI (IAMC-CNR, ITALY), SHAUN S KILLEN (UNIVERSITY OF GLASGOW, AUSTRALIA), MARK I MCCORMICK (JAMES COOK UNIVERSITY, AUSTRALIA), PHILIP L MUNDAY (ARC CENTRE OF EXCELLENCE FOR CORAL REEF STUDIES, AUSTRALIA), MORGAN S PRATCHETT (ARC CENTRE OF EXCELLENCE FOR CORAL REEF STUDIES, AUSTRALIA), SUE-ANN WATSON (ARC CENTRE OF EXCELLENCE FOR CORAL REEF STUDIES, AUSTRALIA)

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Atmospheric carbon dioxide is expected to more than double by the end of the century. The resulting changes in ocean chemistry will affect the behaviour, sensory systems and physiology of many fish species. How these effects may influence the schooling dynamics of gregarious fishes remains poorly understood. In the present study, we examined the influence of elevated CO₂ on the swimming performance, maximum aerobic metabolic rate (MMR) and schooling behaviour in the redbelly yellowtail fusilier (*Caesio cuning*). Schools were acclimated to one of two CO₂ treatments: control (400 μatm) or high-CO₂ (1000 μatm) for two weeks. Schooling dynamics were tested in a 90L swim tunnel system, at four fixed flow speeds: 15, 30, 45 and 60 cm/s. Trials were video-recorded in three dimensions and analysed for school volume, density and shape, the position preference of each individual and their nearest neighbor distance. Individual swimming performance was assessed in terms of gait transition speed (U_{p-c}) and critical swimming speed (U_{crit}) using a continuous acceleration test. MMR was tested using standard respirometry techniques. While individual swimming performance (U_{pc} and U_{crit}) was not impacted by elevated CO₂ conditions, schools exhibited altered swimming behaviour, with reduced position shuffling and cohesion under elevated CO₂ conditions. These results suggest that the tradeoffs of living in a group could be altered under projected future ocean conditions. Given that schooling behaviour is widespread among fishes as a tool for predator avoidance, these changes could have far-reaching consequences for a range of ecologically and economically important fish species.

A8.10 VALIDATING THE USE OF LABORATORY MAINTAINED ANIMALS FOR MACRO-PHYSIOLOGICAL AND MACRO-ECOLOGICAL STUDIES

📅 MONDAY 3 JULY, 2017

🕒 15:25

👤 HEIDI J MACLEAN (AARHUS UNIVERSITY, DENMARK), JESPER G SØRENSEN (AARHUS UNIVERSITY, DENMARK), TORSTEN N KRISTENSEN (AALBORG UNIVERSITY, DENMARK), JOHANNES OVERGAARD (AARHUS UNIVERSITY, DENMARK)

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Large comparative studies in animal ecology, physiology, and evolution often use populations reared in the laboratory for many generations. The relevance of such studies has been criticized because it hinges on the assumption that laboratory populations are representative for their wild living conspecifics. We generated data for paired laboratory maintained and recently caught field populations of nine species of *Drosophila*. We addressed the following two questions: 1) are laboratory maintained and freshly collected animal populations fundamentally different? 2) are data from laboratory maintained animals informative for asking macro-ecological and macro-physiological questions in comparative biology? While we observed a number of significant differences within species between field and laboratory populations, these differences were mostly unsystematic. We show that the between species differences explain the majority of the variance in all traits. Thus clearly demonstrating that differences between species is always more significant than the differences within species. Finally we use these data to demonstrate that macro-physiological and macro-ecological trait correlations are largely similar irrespective if we use trait values from laboratory populations or field populations. Taken together we are able to support the continued use of laboratory maintained populations in comparative studies in ecology, physiology, and evolution.

A8.11 STRUGGLING AGAINST ENTROPY: HOW ION AND WATER HOMEOSTASIS DETERMINE INSECT CHILLING TOLERANCE

📅 MONDAY 3 JULY, 2017

🕒 16:10

👤 HEATH A MACMILLAN (CARLETON UNIVERSITY, CANADA)

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Chill susceptible insects are incapacitated and injured by low temperature exposure before they freeze, but adaptation and acclimation to low temperatures can substantially improve chilling tolerance. Chilling injury is associated with a gradual loss of ion and water homeostasis across cellular membranes and epithelia. This loss of balance occurs most notably across the gut epithelia, where large ionic and osmotic gradients are usually maintained. Dissipation of these gradients at low temperatures causes extracellular [K⁺] levels to rise leading muscle cells to depolarize and initiate apoptosis. Ultimately this issue causes impaired locomotory function and fitness after rewarming. It is unclear whether the root of this cold-induced cascade is a greater net transcellular leak of ions and water in the cold or whether the occluding junctions between

adjacent epithelial cells are disrupted by chilling. It is also unclear whether modifications to transcellular transport or paracellular leak allow insect populations or individuals to adapt or respond to the cold. Here, I will discuss how it is probably both; plasticity in the transcellular transport properties and paracellular permeability of the renal epithelia drive the wide variation in chilling tolerance observed both within and among *Drosophila* species. In particular, changes to the expression of major structural proteins of the septate junctions and the activity of ion-motive ATPase enzymes (which energize the secretion and reabsorption of ions and water in the Malpighian tubules and gut epithelia) underlie both cold acclimation in *Drosophila melanogaster*, and variation in cold tolerance among *Drosophila* species.

A8.12 THE PHYSIOLOGY OF INSECT CHILL INJURY: COLD INDUCED DEPOLARIZATION OF CELL POTENTIAL CAUSES CHILL INJURY THROUGH LOSS OF INTRACELLULAR Ca^{++} REGULATION IN LOCUST MUSCLE CELLS

MONDAY 3 JULY, 2017 16:40

JOHANNES OVERGAARD (AARHUS UNIVERSITY, DENMARK)

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Cold tolerance of insects is critically important for their ability to persist in temperate and arctic environments. Little is known, however, of the proximal causes leading to cold injury in this animal group. Exposure to low temperature has been shown to coincide with depolarization of excitable tissue in many insects. This depolarization is caused by a decreased active ion transport and by loss of transmembrane ion-balance. Although the development of severe depolarization is closely correlated to the occurrence of chill injury it is not known if there is a direct causative link between these co-occurring phenomena. To investigate this further we use a fluorescent dual DNA staining method to estimate cell viability in muscle fibers from the migratory locust (*Locusta migratoria*). We then exposed the *in vitro* muscle cell preparations to depolarizing conditions using either hypothermia, hyperkalemia or pharmacological interventions and found that cellular injury develops regardless of the treatment causing loss of cell potential. We speculate that this depolarization induced injury is caused by activation of voltage sensitive Ca^{++} channels that disrupts intracellular Ca^{++} balance. This hypothesis was supported by a series of experiments in which the cells would avoid development of chill injury if Ca^{++} is removed or if Ca^{++} channels are blocked. In conclusion: Cellular cold injury in insects is caused by loss of Ca^{++} regulation that is directly related to activation of voltage dependent Ca^{++} channels during cold stress.

A8.16 EFFECT OF DISPERSANT-TREATED OIL ON THE BEHAVIOUR OF THE EUROPEAN SEA BASS, *DICENTRARCHUS LABRAX*

MONDAY 3 JULY, 2017 16:55

CASSANDRE AIMON (UNIVERSITÉ DE BRETAGNE OCCIDENTALE, FRANCE), NICOLAS LE BAYON (IFREMER, FRANCE), STÉPHANE LE FLOCH (CEDRE, FRANCE), GUY CLAIREAUX (UNIVERSITÉ DE BRETAGNE OCCIDENTALE, FRANCE)

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Anthropic activities induce several environmental disturbances including chemical pollution. Although their occurrence has decreased over the last 30 years, oil spills are still highly relevant in this regards. In fish, oil exposure has physiological effects that vary according to exposure duration, timing of the assessment, species, life stage or nature of the oil. Some studies have shown that polycyclic aromatic hydrocarbons, a major component of oil, can disturb the neurosensory capacities of fish and are, therefore, liable to affect their behavioural repertoire, causing improper use of their habitat. This aspect of an oil spill is, however, poorly studied.

In the present experiment, we investigated, in a dose-response manner, the influence of a 48h-oil exposure on sea bass neophobia and boldness using an open field test. Three different oil concentrations were tested (0.80 g L^{-1} , 0.40 g L^{-1} and 0.17 g L^{-1}) and compared to a control. Fish behaviour was tested during the 3 weeks following oil-exposure. Preliminary results indicate that at the introduction in the arena, the exposed fish did not show the commonly observed inhibition period in regards to novelty. They were swimming at a constant speed and spent most of the time in the central area of the arena. Except for the most severe treatment, fish totally recover normal behaviour after 3 weeks. These findings show that exposition to chemically dispersed oil temporally decreased neophobia and danger consciousness.

A8.22 MATERNAL ANTIGEN EXPOSURE ENHANCES IMMUNITY AND INCREASES METABOLIC RATE IN NESTLING TREE SWALLOWS

MONDAY 3 JULY, 2017 17:10

GARY BURNES (TRENT UNIVERSITY, CANADA), DEANNA MOHER (TRENT UNIVERSITY, CANADA), NOAH BEN-EZRA (TRENT UNIVERSITY, CANADA), RYAN J KELLY (TRENT UNIVERSITY, CANADA), DENNIS HASSELQUIST (LUND UNIVERSITY, SWEDEN), EUNICE H CHIN (TRENT UNIVERSITY, CANADA)

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Female birds transfer maternal antibodies (matAb) to their nestlings, via the egg. To test whether there is an energetic benefit to nestlings of receiving matAb, we challenged adult female tree swallows (*Tachycineta bicolor*) prior to clutch initiation with either lipopolysaccharide (LPS) or saline (Control). Following hatching of eggs, one half of each adult female's nestlings were then immunized with either LPS or saline, and for 4-hrs post

immunization nestling metabolic rate (MR) was measured. There was an interaction between maternal and nestling treatment on nestling MR, driven by an increase in the MR rate of control nestlings from immunized mothers. Near fledging, we challenged all nestlings with phytohaemagglutinin (PHA) to test the impact of mat Ab on the ability of nestlings to mount a subsequent immune response. Nestlings from LPS-mothers had a greater response to PHA than did nestlings from Control-mothers. There was also a trend for LPS-mothers to have higher fledging success than Control-mothers. Females in both treatments had similar probabilities of returning to breed the following year. Our data thus suggest potential benefits of maternal antibody transfer to mothers and offspring. Although nestlings of immunized mothers had increased energy requirements, this was presumably met by increased parental feeding workload, with no negative impact on maternal survival.

A8.19 EFFECT OF AMBIENT TEMPERATURE ON SPONTANEOUS LOCOMOTOR ACTIVITY AND DAILY ENERGY EXPENDITURE IN MICE DIVERGENTLY SELECTED FOR HIGH AND LOW BASAL METABOLIC RATE

📅 MONDAY 3 JULY, 2017 ⌚ 17:25

👤 PAWEŁ BRZEK (UNIVERSITY OF BIALYSTOK, POLAND), ANDRZEJ GEBCZYŃSKI (UNIVERSITY OF BIALYSTOK, POLAND), JULITA SADOWSKA (UNIVERSITY OF BIALYSTOK, POLAND), ANETA KSIAZEK (UNIVERSITY OF BIALYSTOK, POLAND), JAN NEDERGAARD (STOCKHOLM UNIVERSITY, SWEDEN), MAREK KONARZEWSKI (UNIVERSITY OF BIALYSTOK, POLAND)

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High basal metabolic rate (BMR) may provide several evolutionary benefits. However, it can potentially become disadvantageous under both high (problems with heat dissipation) or low (higher food demands) ambient temperatures. We compared spontaneous physical activity (SPA) and daily energy expenditure (DEE) in laboratory mice from artificial selection for high (H-BMR) or low (L-BMR) BMR maintained at 23°C or subjected to long-term exposure to either high (30°C) or low (4°C) ambient temperature. In accordance with our earlier experiments, H-BMR mice showed higher SPA intensity. Long-term exposure to 30°C reduced SPA intensity but it remained higher in H-BMR than in L-BMR mice. Difference between lines in total SPA disappeared after long-term exposure to 4°C but was elevated during short (1 day) exposure. H-BMR mice had higher DEE than L-BMR but this difference was smaller at 23°C than at 30°C and became non-significant in mice exposed to 4°C. This pattern agrees with our earlier results, indicating that selection for high BMR widened the thermoneutral zone of mice. We conclude that positive correlation between BMR and SPA is maintained when ambient temperature increases. At the same time, heat generated by high BMR substitutes the need for specific thermogenesis, and thus decreases relative cost of high BMR in lower ambient temperature. We discuss potential implications of these results for ongoing studies of capacity of non-shivering thermogenesis in mice selected for high and low BMR. Financial support: National Science Centre, Poland, grant 2014/15/B/NZ8/00244 for B.P.

A8.15 CO-EXISTENCE WITH NON-NATIVE BROOK TROUT DISRUPTS THE INTEGRATION OF PHENOTYPIC TRAITS IN BROWN TROUT

📅 THURSDAY 6 JULY, 2017 ⌚ 13:50

👤 JÖRGEN I. JOHANSSON (UNIVERSITY OF GOTHENBURG, SWEDEN), LIBOR ZÁVORKA (CNRS UNIVERSITÉ DE TOULOUSE, FRANCE), BARBARA KOECK (UNIVERSITY OF GLASGOW, UNITED KINGDOM), JULIEN CUCHEROUSSET (CNRS UNIVERSITÉ DE TOULOUSE, FRANCE), JEROEN BRIJJS (UNIVERSITY OF GOTHENBURG, SWEDEN), JOACIM NÅSLUND (UNIVERSITY OF SOUTH BOHEMIA, CZECH REPUBLIC), DAVID ALDVÉN (VATTENFALL AB, SWEDEN), JOHAN HÖJESJÖ (UNIVERSITY OF GOTHENBURG, SWEDEN), IAN A. FLEMING (MEMORIAL UNIVERSITY OF NEWFOUNDLAND, CANADA)

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Invasive species can induce novel environmental pressures affecting all levels of biological organization, from genes to ecosystem. However, the extent to which invasive species affect the association among phenotypic traits in native species is currently unknown. Here, field monitoring in a natural stream was combined with estimates of behavioral, physiological and morphological traits to address the hypothesis that coexistence with a non-native invader induces a novel environmental pressure that disrupts the adaptive integration among phenotypic traits of the native species. We compared the integration among key phenotypic traits (i.e. aerobic scope, standard metabolic rate, body growth, activity, and body shape) and ecological niche traits (i.e. spring and summer diet, home range size, daily movements) of an allopatric group of native brown trout (*Salmo trutta*) with a group of brown trout living sympatrically with non-native brook trout (*Salvelinus fontinalis*). We found that the integration of phenotypic traits was substantially reduced in sympatric brown trout. Furthermore, allopatric and sympatric brown trout differed in key phenotypic and ecological niche traits where brown trout living in sympatry with non-native brook trout ate more terrestrial prey, had smaller home ranges, a stouter body shape and lower growth rate than allopatric brown trout. These results are generally consistent with our hypothesis suggesting that the reduction in fitness (i.e. growth rate) observed in sympatric brown trout is caused by the breakdown of their adaptive phenotypic syndrome. Our results may help explaining deleterious effects of non-native species reported in the absence of direct competition with the native species.

A8.13 FROM BEYOND THE GRAVE: DOES THE NECROBIOME IMPACT METABOLIC PERFORMANCE OF RAINBOW DARTERS (*ETHEOSTOMA CAERULEUM*) DOWNSTREAM OF WASTEWATER EFFLUENT OUTLETS?

THURSDAY 6 JULY, 2017 14:05

PAUL M CRAIG (UNIVERSITY OF WATERLOO, CANADA), ANDREW C DOXEY (UNIVERSITY OF WATERLOO, CANADA), MIKE LYNCH (UNIVERSITY OF WATERLOO, CANADA), RHIANNON HODGSON (UNIVERSITY OF WATERLOO, CANADA), NICOLE CSONKA (UNIVERSITY OF WATERLOO, CANADA)

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Aquatic organisms are continuously exposed to multiple stressors, such as heat, low oxygen, and elevated carbon dioxide, which cumulatively result in significant impacts on metabolic performance in aquatic species. Exacerbating these effects are the anthropogenic influences of municipal wastewater effluent discharged into local rivers containing a complex mixture of residential, industrial, and institutional waste. The major chemical components of effluent include endocrine disruptors, pharmaceuticals, and personal care products, which have been demonstrated to elicit a dramatic decrease in rainbow darter fitness, including severe intersex, reduced gonad size, and decreased androgen production. Adding further insult is the enrichment of pathogenic bacteria derived from wastewater effluent. This study examines the metabolic performance of rainbow darters in clean and effluent influenced waters from the Grand River watershed in combination with the impact of pathogenic bacteria derived from the necrobiome. We demonstrate that the necrobiome, defined as the microbial population derived from dead and decaying organisms, is heavily influenced by the life history of the rainbow darter exposed to wastewater effluent. Necrobiome enrichment discovered a number of pathogenic species, such as *Clostridium perfringens*, *Aeromonas veronii*, and *Eubacterium tarantellus*, all of which are implicated in performance reducing morbidity phenotypes. We found fish exposed to wastewater effluent had a significant reduction in aerobic scope and associated enzymatic parameters implying a distinct impact on performance indices. To our knowledge, this is the first study to examine the influence of the necrobiome on the associated metabolic performance of living fish in wastewater effluent influenced waters.

A8.17 THE SAILFISH HUNTING STRATEGY: THE ADVANTAGES OF BEING GREGARIOUS PREDATORS

THURSDAY 6 JULY, 2017 14:20

STEFANO MARRAS (NATIONAL RESEARCH COUNCIL - CNR, ITALY)

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Billfishes are large pelagic predators that prey on schooling fishes. Field studies on sailfish (*Istiophorus platypterus*) hunting on schooling sardines (*Sardinella aurita*) were performed off the Yucatan Peninsula (Mexico) to characterize the mechanisms underlying predatory success. Previous work suggested that sailfish reach extremely high speeds, up to 35 ms^{-1} . However, high-speed video during hunting, accelerometry and measures of minimum muscle contraction times, revealed that sailfish swim at speeds up to about 8 ms^{-1} . To hunt successfully, sailfish typically insert their bills into a school of prey. Interestingly, the prey do not show any startle response at this stage, suggesting that the bill acts as a 'stealthy object' causing little disturbance. Subsequently, the bill is rotated with a rapid slashing maneuver to injure the prey, thereby making them more likely to be captured. Sailfish also use a primitive form of cooperation ('proto-cooperation') whereby individuals take advantage of group behavior, thus increasing their chances of catching prey. Furthermore, each individual sailfish is lateralized, i.e. specialized to rotate its bills in one specific direction (right or left) when slashing prey, although lateralization does not occur at the population level. Therefore, sailfish exploit the advantages of a lateral specialization, without the disadvantages of becoming predictable in their attacks, since the slashing direction of individuals within a group of sailfish will not be predictable to the prey. This provides novel insights into the evolution of lateralization at the individual level in gregarious predators.

A8.18 CONFLICT IN MUTUALISTIC INTERACTIONS MAINTAINS HIGH ESCAPE PERFORMANCE IN THE CLEANER FISH *LABROIDES DIMIDIATUS*

THURSDAY 6 JULY, 2017 14:35

DOMINIQUE G ROCHE (UNIVERSITY OF NEUCHÂTEL, SWITZERLAND), SIMON GINGINS (UNIVERSITY OF NEUCHÂTEL, SWITZERLAND), REDOUAN BSHARY (UNIVERSITY OF NEUCHÂTEL, SWITZERLAND)

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Predatory reef fishes regularly visit mutualistic cleaner fish (*Labroides dimidiatus*) to get their ectoparasites removed but show no interest in eating them. The concept of compensated trait loss posits that characters can be lost if a mutualistic relationship reduces the need for a given trait. Thus, selective pressures on escape performance might have relaxed in *L. dimidiatus* due to its privileged relationship with predators. However, the cost of failing to escape a predatory strike is extreme even if predation events on cleaners are exceptionally rare. Additionally, cleaners must escape from non-predatory clients that regularly punish them for eating mucus

instead of parasites. Therefore, strong escape capabilities might instead be maintained in cleaner fish because they must be able to flee when in close proximity to predators or dissatisfied clients. We compared the fast-start escape performance of *L. dimidiatus* with that of five closely related wrasse species and found that the mutualistic relationship that cleaners entertain with predators has not led to reduced escape performance. Instead, conflicts in cleaning interactions appear to have maintained selective pressures on this trait, suggesting that compensated trait loss might only evolve in cases of high interdependence between mutualistic partners that are not tempted to cheat.

A8.20 DELETERIOUS IMPACT OF HIF1 α KNOCKOUT ON HYPOXIA PERFORMANCE IN LARVAL ZEBRAFISH (*DANIO RERIO*)

THURSDAY 6 JULY, 2017 14:50

MILICA MANDIC (UNIVERSITY OF OTTAWA, CANADA),
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A multitude of traits across levels of biological organization underlie hypoxia performance, a complex phenotype. The coordination of these traits is partly attributed to hypoxia inducible factor 1 α (HIF1 α), a transcription factor considered to be one of the essential controllers of the hypoxic stress response in vertebrates. What is not known is the degree of HIF1 α contribution to hypoxia performance and the specific genes altered by HIF1 α that produce large effect traits underlying hypoxia performance. In cyprinids there are two paralogs of HIF1 α (HIF1 α -aa and HIF1 α -ab) and in this study we examined hypoxia performance in wild-type, HIF1 α -aa knockout, HIF1 α -ab knockout and double knockout of both paralogs in larval zebrafish (*Danio rerio*). Specifically, we assessed critical O₂ tension (P_{crit}) in 7 days post fertilization (dpf) larva raised under normoxia, mild chronic hypoxia (3 days of 90 mmHg starting at 4 dpf) and severe sub-chronic hypoxia (1 day of 30 mmHg starting at 6 dpf). Double knockout of both paralogs resulted in higher P_{crit}, indicating lower hypoxia performance, than wild-type zebrafish raised under normoxia. Exposure to severe, sub-chronic hypoxia improved hypoxia performance (decreased P_{crit}) in both wild-type and double knockout larvae, while exposure to mild chronic hypoxia caused an enhancement of hypoxia performance in wild-type larvae only. There was no difference in P_{crit} between HIF1 α -ab knockout and wild-type, in contrast to HIF1 α -aa knockout which produced similar phenotypic differences as the double paralog knockout. These results indicate that only one paralog, HIF1 α -aa, has a significant effect on hypoxia performance in developing zebrafish larvae.

A8.21 EFFECTS OF CAROTENOIDS ON THE COST OF REPRODUCTION TO A LIVE-BEARING FISH

THURSDAY 6 JULY, 2017 15:45

DANIEL SANCHEZ-LACALLE (UNIVERSITY OF THE WEST OF SCOTLAND, UNITED KINGDOM), DONNA L SNELLGROVE (WALTHAM CENTRE FOR PET NUTRITION, UNITED KINGDOM), KATHERINE A SLOMAN (UNIVERSITY OF THE WEST OF SCOTLAND, UNITED KINGDOM)

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Chronic activities which increase metabolic rate, such as gestation, result in an increase in production of reactive oxygen species (ROS), increasing the risk of oxidative damage. Dietary carotenoids have been shown to play a role in protecting against oxidative stress. It was hypothesized that dietary carotenoids would help to reduce the metabolic costs associated with breeding in a live bearing fish. Guppies (*Poecilia reticulata*) were fed either a high (216 mg kg⁻¹) or low (40 mg kg⁻¹) carotenoid diet and divided into breeders and non-breeders. Sustained swimming performance was measured as an indirect measurement of oxidative damage/fitness. Prior to breeding, after 6 weeks on the experimental diets, males fed the high carotenoid content diet swam for longer. After 12 weeks on the diet and after breeding groups were allowed to breed, there was no effect of diet on swimming performance but non-breeders were able to swim for longer than breeders and females were able to swim for longer than males. In conclusion, breeding had an effect on swimming performance of guppies, and although a high carotenoid diet improved performance of males prior to breeding, carotenoids did not reduce the impact of breeding. Identifying dietary components that can help reduce the impact of breeding could improve pet fish health and welfare within the ornamental fish trade.

A8.14 THE ENVIRONMENT INDUCES COMPLEX AND DYNAMIC ALTERATIONS OF THE INTESTINE AND OTHER TISSUES DURING DIGESTION

THURSDAY 6 JULY, 2017 16:00

CAROL BUCKING (YORK UNIVERSITY, CANADA), LEAH TURNER (YORK UNIVERSITY, CANADA), PRANAV DHAKAL (YORK UNIVERSITY, CANADA), TAYLOR SPARRING (YORK UNIVERSITY, CANADA), MELANIE WILLIAMS (YORK UNIVERSITY, CANADA)

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Digestion is a dynamic process with wide-ranging effects on systemic physiology and the environment plays an important part in determining the impacts digestion exerts. Integrative and comparative studies will reveal diverse, plastic responses to digestion across fish species. In particular, the gastrointestinal tract plays a co-ordinated role in piscine osmo- and iono-regulatory homeostasis, as well as nitrogen balance, with multiple organ systems such as the gill and kidney. During digestion, this

role is augmented and the resulting complex interactions are discussed. Furthermore, integration across biological levels within the gastrointestinal tract itself has exposed multiple levels of variable responses within enterocytes during digestion. The integrated responses of the intestine to environmental and dietary manipulations will be highlighted. Using stenohaline and euryhaline models, as well as eurythermal models, we've uncovered several impacts of the environment (salinity and temperature) on digestion.

A8.23 DEFENSE IS COSTLY - IMMUNE STIMULATION INCREASES THE METABOLISM OF FISH

📅 THURSDAY 6 JULY, 2017 ⌚ 16:15

👤 MALIN ROSENGREN (UNIVERSITY OF GOTHENBURG, SWEDEN), EMILIA BENAVENTE NORRMAN (UNIVERSITY OF GOTHENBURG, SWEDEN), FREDRIK JUTFELT (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY), LARS NIKLASSON (UNIVERSITY OF GOTHENBURG, SWEDEN), KRISTINA SUNDELL (UNIVERSITY OF GOTHENBURG, SWEDEN)

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While the adaptive value of immune-suppressive action during e.g. long term stress is argued to stem from competition for energy, experimental studies verifying this are scarce. The present study therefore investigated the effects of the innate immune response on standard metabolic rate (SMR) and swim performance of rainbow trout. This was performed by exposing fish to bacterial and viral mitogens (LPS and PolyI:C), which triggers the immune system similarly to an infection, while excluding energetic costs induced by e.g. pathogen proliferation and tissue repair. Fish treated with immune stimulants showed a 10% higher SMR, which was sustained for 5 days post-injection, compared to controls. The immune stimulated groups also showed higher expressions of the pro-inflammatory cytokine IL-1 β and of Mx proteins in the head kidney, verifying an active immune response. This metabolic cost could compromise other energetically demanding activities, such as swimming. We therefore hypothesized that the maximum swimming speed would be negatively affected following immune stimulation. Using a Brett-type swim tunnel, a 1,5 h swim protocol revealed a tendency for a lower maximum swim speed in the LPS group, however, no overall significant difference was found. The higher SMR levels in the immune stimulated groups show that even in the absence of metabolic fever and pathogen activity, inflammatory processes alone will increase energetic demand. This could lead to tertiary negative effects on growth and reproduction and the results supports the notion that immune activity is suppressed during stressful challenges due to competition for energy.

A8.24 TILAPIA GUT MICROBIOME IN RESPONSE TO TEMPERATURE AND COLD ADAPTATION

📅 THURSDAY 6 JULY, 2017 ⌚ 16:30

👤 FOTINI KOKOU (BEN GURION UNIVERSITY OF THE NEGEV, ISRAEL), AVNER CNAANI (AGRICULTURAL RESEARCH ORGANIZATION, ISRAEL), ITZHAK MIZRAHI (BEN GURION UNIVERSITY OF THE NEGEV, ISRAEL)

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Cold is an environmental challenge that affects metabolic processes thus adaptations in response to acute challenges or temperature fluctuations are expected to be found across the tree of life. When exposed to cold temperatures, mammals undergo physiological adaptations and recent work revealed that intestinal microbes facilitated these key adaptations. This is not surprising as studies have proven so far, the importance of the gut microbial communities for host functioning. On the other hand, these communities need to adapt to several environmental factors which lead to 'environmental filtering'. Poikilothermic animal physiology is strongly influenced by temperature, thus we hypothesize that also their microbiota is affected, but so far experimental studies exploring how temperature affects the interactions between both parties are rare. To this end, we examined for the first time the effects of cold exposure on the gut microbiome of the Blue tilapia, one of the most cold-tolerant tilapia species. We have used fish for enhanced cold tolerance. Sensitive and cold-tolerant fish were challenged for cold tolerance at 12°C. For control, we used fish originating from the same families kept at the optimal temperature of 24°C. At the end of the trial, we sampled the intestine and performed sequencing for the 16S ribosomal RNA as a marker for microbial diversity. Our results suggest for the first time in fish that temperature is a major driving force shaping the gut microbiome acting as an environmental filter and such changes in microbial dynamics will be discussed in the context of host physiology.

A8.25 CHILL SUSCEPTIBILITY OF THE INSECT CENTRAL NERVOUS SYSTEM: A COMPARATIVE STUDY OF TEMPERATE AND TROPICAL *DROSOPHILA*

📅 THURSDAY 6 JULY, 2017 ⌚ 16:45

👤 MADS K ANDERSEN (AARHUS UNIVERSITY, DENMARK),
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Temperature is one of the most important abiotic factors determining function and performance in insects. At critically low temperatures, the majority of insects enter a reversible state of paralysis where an initial loss of coordinated movement develops to a complete cessation of muscle activity. The cause of these events has been linked to loss of function in either the central nervous system (CNS) or the muscular system caused by cold-induced depolarization of these excitable tissues. For *Drosophila* it was recently found that coma coincided with muscle depolarization in some cold-sensitive species while other cold-hardy species did not experience this muscle depolarization in relation to coma. To investigate this further we examined the role of the CNS in relation to CT_{min} in the same five species of *Drosophila*. During gradual cooling, we observed in all species a spreading depolarization (SD), which silences the CNS. Further we found that the species-specific temperature causing SD events correlated strongly with behavioural CT_{min} . SD events were always associated with a surge in extracellular K^+ concentration in the CNS and we found that cold-sensitive species experienced surges with larger amplitude than their cold-hardy allo-species. Finally we show that CNS function of cold-tolerant species are more resilient to anoxia suggesting that they have an increased capacity to defend ion homeostasis in the CNS in the absence of active ion transport. In combination these results suggest that differences in homeostatic capacity of the CNS underlie the interspecific differences in CT_{min} of *Drosophila*.

A8.26 TEMPERATURE DEPENDENT BETWEEN- AND WITHIN-INDIVIDUAL VARIATION IN BEHAVIOUR IN WILD ZEBRAFISH (*DANIO RERIO*)

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 METTE H FINNOEN (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY), JONATHAN WRIGHT (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY),
FREDRIK JUTFELT (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY), RACHAEL L. MORGAN (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY)

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Zebrafish are found in a wide range of temperatures, and are a highly tolerant species. We investigated the plasticity and between-individual differences in zebrafish behaviour at two temperatures below and two temperatures above their laboratory housed temperature of 28°C. We were interested in exploring unexplained within-individual variance (heterogeneous residuals), which is not well integrated into evolutionary theory. We specifically tested whether the heterogeneous residuals were caused by (1) a non-linear reaction norm (i.e., non-linear plasticity), (2) passive plasticity where physical processes like temperature create phenotypic variation, or (3) a breakdown of plasticity at the extreme ends of an environmental variable. The latter suggests that an individual's level of aggression at a specific temperature remains constant if the temperature is within a specific range. However, at a temperature outside of this range an individual's level of aggression varies considerably. This suggests that ectothermic organisms, such as the zebrafish, can express the desired level of a behaviour despite fluctuations in the environmental temperature. It is when the change in temperature is too large that the fish are not able to maintain the desired level of a behaviour, leading to the possibly harmful inconsistent expression of behaviour.

A8.27 RESPONSE OF EVAPORATIVE WATER LOSS RATE AND THERMAL PREFERENCE TO DEHYDRATION IN TWO LIZARDS FROM DIFFERENT HABITATS AT HIGH ALTITUDES

TUESDAY 4 JULY, 2017 POSTER SESSION

KUAN-WEI HUNG (NATIONAL SUN YET-SEN UNIVERSITY, TAIWAN), TE-EN LIN (ENDEMIC SPECIES RESEARCH INSTITUTE, TAIWAN), SHU-PING HUANG (NATIONAL SUN YET-SEN UNIVERSITY, TAIWAN)

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An increase in temperature and aridity caused by current climate change has impacted many species. Physiological and behavioral traits have been increasingly used in mechanistic models to predict species response to changing environments, which provide vital information for wildlife management. In reptiles, the preferred body temperature (T_p) and evaporative water loss (EWL) are often used to simulate their thermal niche in the field. However, very few studies have considered that reptiles may select lower temperatures during water stress in the field. Taking T_p of lizards only in the hydrated state may lead to an inaccurate prediction of thermal niches. We postulate that lizards' T_p and EWL are correlated to their habitat microclimate and T_p is adjustable for water conservation during times of water stress. We test this hypothesis using two reptiles from high-elevation areas (>2000m) in Taiwan: a skink *Sphenomorphus taiwanensis* inhabiting cool, humid forest edges, and a grass lizard *Takydromus hsuehshanensis* inhabiting warm, open grasslands. The results showed that their T_p and EWL reflect well the respective habitat microclimate. When in water stress, *T. hsuehshanensis* selected a lower T_p in a thermogradient, while *S. taiwanensis* did not change its T_p . Neither species exhibited physiological means for water conservation as their EWL rates did not change significantly during 2-7 consecutive days of dehydration when they lost 15% - 20% of body mass. These results support our hypothesis and suggest that species-specific phenotypic plasticity of T_p should be considered in forecasting species response to changing environments, particularly in water stress conditions.

A8.28 SCHOOLING OF PACIFIC SARDINES (*SARDINOPS SAGAX*) UNDER EXPERIMENTAL HYPOXIA

TUESDAY 4 JULY, 2017 POSTER SESSION

NICHOLAS CAREY (HOPKINS MARINE STATION STANFORD UNIVERSITY, UNITED STATES), JEREMY A GOLDBOGEN (HOPKINS MARINE STATION STANFORD UNIVERSITY, UNITED STATES), PAOLO DOMENICI (IAMC-CNR ISTITUTO PER L'AMBIENTE MARINO COSTIERO, ITALY)

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Fish schooling is important in lowering energetic costs of swimming through hydrodynamic effects, in reducing the need for individual vigilance, and in protecting against predation through confusing potential predators. Climate change will cause more frequent and extensive low oxygen zones in future oceans, particularly in upwelling areas such as the eastern Pacific. These hypoxic zones are likely to affect the energetics of commercially important fish species and their schooling behaviour, as previously shown in some species. We set out to test if hypoxia will cause loss of school cohesion or changes in behaviour in a pelagic schooling forage fish, the Pacific sardine *Sardinops sagax*. This species occurs in vast schools which support a range of predator communities, and are an important commercial stock in the region. Small schools (7 individuals) were filmed under normoxia in a circular raceway which they circled in schooling form. They were then experimentally exposed to progressive hypoxia (<20% air saturation) and again filmed for several hours. Videos were analysed for school structure, including shape, distance between individuals, and frequency of positional changes. Unlike previous work on the effect of hypoxia on schooling which did not keep track of individual fish, here, the unique body patterning of the sardines allowed such tracking. We were therefore able to determine the position of each fish over time, which allowed us to test the likelihood of individuals to be leaders or followers within the school and whether these positional preferences were affected by hypoxia.

A8.29 NEMATODE INFECTION, SWIMBLADDER FUNCTION AND THE SPAWNING MIGRATION OF THE EEL

TUESDAY 4 JULY, 2017 POSTER SESSION

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The nematode *Anguillicola crassus* was introduced to Europe in the early 1980-ies and spread all over Europe within 10 years. As a histophagous parasite *Anguillicola* lives in the swimbladder and significantly impairs gas secretion. Illumina RNAseq data demonstrated that the infection of the swimbladder provoked a strong immune response, in particular in yellow eels. Enzyme activities and transcriptional studies also revealed significant modifications of metabolic pathways critical for swimbladder function like glycolysis, ROS defense,

and the formation of the extracellular matrix. The negative influence of the parasitic nematode on various metabolic pathways was much stronger in yellow eels as compared to silver eels. The severe impact of the nematode on swimbladder function is expected to significantly impair a successful spawning migration of the European eel to the Sargosso Sea.

Financial support was granted by the Austrian Science Foundation FWF: P26363-B25.

A8.30 SLOW AND STEADY SECURES SURVIVAL: HOW DIFFERENCES IN EPITHELIAL K^+ TRANSPORT UNDERLIE INTERSPECIFIC DIFFERENCES IN *DROSOPHILA* COLD TOLERANCE

TUESDAY 4 JULY, 2017 POSTER SESSION

MADS K ANDERSEN (AARHUS UNIVERSITY, DENMARK), HEATH A MACMILLAN (YORK UNIVERSITY, CANADA), ANDREW DONINI (YORK UNIVERSITY, CANADA), JOHANNES OVERGAARD (AARHUS UNIVERSITY, DENMARK)

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The majority of insects succumb to chill injury at low temperatures above those that cause them to freeze. Chill tolerance is strongly associated with the ability to maintain ion and water homeostasis during cold exposure. Maintenance of K^+ balance is particularly important due to its role in setting the membrane potential. In most insects, K^+ balance is maintained through active secretion to balance reabsorption from the hindgut and passive leak arising from the gut lumen. Here, we used a scanning ion-selective electrode technique (SIET) system at high (23°C) and low (6°C) temperature to examine K^+ flux across the major ion regulatory epithelia (the Malpighian tubules and the hindgut) in five *Drosophila* species with different chill tolerance. We found that chill susceptible species secreted less K^+ from the Malpighian tubules at low temperatures as opposed to their cold-adapted allopecifics that were able to defend transport rates at low temperature. Furthermore we found that low temperature increased K^+ reabsorption in the hindgut in chill susceptible species, whereas cold-adapted species lowered reabsorption rates. Preliminary analysis suggests that increased reabsorption rates in chill susceptible species may be an artefact from increased leakage from the gut during cold-exposure. In conclusion, cold-adapted *Drosophila* are better at maintaining K^+ homeostasis through an increased ability to maintain K^+ secretion rates and through reduced leakage towards the hemolymph. These adaptations ensure that cold-adapted species experience less perturbation of K^+ homeostasis during cold stress while warm-adapted species succumb to chill injury caused by detrimental K^+ loading into the hemolymph.

A8.31 THE SPECIALIST-GENERALIST MODEL OF BODY TEMPERATURE REGULATION DOES NOT DEPEND ON SEX DIFFERENCES IN HETEROTHERMY USE

TUESDAY 4 JULY, 2017 POSTER SESSION

ANNA S PRZYBYLSKA (NICOLAUS COPERNICUS UNIVERSITY, POLAND), JAN S BORATYŃSKI (MUSEUM AND INSTITUTE OF ZOOLOGY POLISH ACADEMY OF SCIENCES, POLAND), MICHAŁ S WOJCIECHOWSKI (NICOLAUS COPERNICUS UNIVERSITY, POLAND), MAŁGORZATA JEFIMOW (NICOLAUS COPERNICUS UNIVERSITY, POLAND)

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Body temperature (T_b) of homeothermic animals varies on daily and seasonal basis. The degree of these variations increases with increasing environmental demands, like food deprivation and low ambient temperature. According to theoretical predictions, endothermic homeotherms can be classified as either thermal specialists which regulate their T_b precisely, or generalists which tolerate greater variations of T_b . In high cost environments, thermal specialists are supposed to be more prone to use facultative heterothermy than generalists. We tested this hypothesis on the intraspecific level using laboratory mice (C57BL/cmdb). We used both sexes, as they differ in propensity to use torpor in response to increased energy demands. We measured T_b and metabolic rate of 8 male and 7 female mice that were fasted for 48h at 20°C. In response to food deprivation, females entered long and deep torpor bouts, while males showed only shallow heterothermic episodes. As a result, females had significantly higher heterothermy index (HI) than males. Moreover, we found that animals with more precise thermoregulation (more constant T_b) when fed, had more variable T_b when fasted, and this was true for both, males and females. Our results suggest that the thermoregulatory specialist-generalist trade-off is a continuum that includes a wide spectrum of thermal sensitivities, and can be applied on the intraspecific level.

A8.33 NON-SHIVERING THERMOGENESIS DOES NOT INCREASE PLASMA REACTIVE OXYGEN METABOLITES LEVEL BUT AUGMENTS ANTIOXIDANT POTENTIAL IN WINTER-ACCLIMATED SIBERIAN HAMSTERS

TUESDAY 4 JULY, 2017 POSTER SESSION

MICHAŁ S WOJCIECHOWSKI (NICOLAUS COPERNICUS UNIVERSITY, POLAND), ANNA S PRZYBYLSKA (NICOLAUS COPERNICUS UNIVERSITY, POLAND), MAŁGORZATA JEFIMOW (NICOLAUS COPERNICUS UNIVERSITY, POLAND)

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Facultative non-shivering thermogenesis (fNST) is a main source of heat in cold acclimated small mammals. It is often assumed that large increase in metabolic rate during intense heat production during normothermy or during arousal from torpor induces oxidative stress. We examined this prediction in Siberian hamster (*Phodopus sungorus*), a small heterothermic rodent, which seasonally increases fNST capacity and develops the ability to enter torpor. We measured fNST capacity as an increase in metabolism after noradrenaline (NA) injection in 67 winter-acclimated hamsters (35 males and 32 females); in 20 hamsters we also measured heat production after saline injection. Immediately after calorimetry trials we took a blood sample from each animal and measured concentration of reactive oxygen metabolites (ROM, $\text{mgH}_2\text{O}_2 \text{dL}^{-1}$) and biological antioxidant potential (BAP, $\mu\text{mol vitamin CL}^{-1}$) in plasma. We found that neither ROM production nor BAP correlated with fNST capacity ($p > 0.05$). ROM concentration after NA and saline injections also did not differ ($p > 0.05$). However, BAP was approximately 15% higher after fNST induction than after control injection. These results suggest that animals which routinely experience large variations in heat production, evolved effective mechanisms protecting them from potentially detrimental effects of profound changes in metabolism. The study was supported by the grant #NCN2014/13/B/NZ8/04698.

A8.34 LOCOMOTOR PHYSIOLOGY OF A HIBERNATING FISH IN THE FAMILY LABRIDAE

TUESDAY 4 JULY, 2017 POSTER SESSION

CLINTON J MORAN (FAIRFIELD UNIVERSITY, UNITED STATES), SHANNON P GERRY (FAIRFIELD UNIVERSITY, UNITED STATES)

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Winter conditions in the Northwest Atlantic cause cunner (*Tautoglabrus adspersus*) to enter a state of extended torpor when water temperatures drop below 10°C . As one of the northernmost species in the primarily tropical family, Labridae, this species has adopted a form of hibernation as a physiological response to unfavorable thermal conditions. We examined the impact of acclimation temperature (5, 10, 15, 20°C) on steady swimming and muscle performance in cunner. We hypothesized that maximum sustainable swimming speed and gait transition speed would increase with increasing temperature. Additionally, muscle power output will significantly increase with increasing temperature. Through swimming step trials we found that maximum sustainable swimming speed and gait transition speed increased significantly with increasing temperature. Increased swimming performance with increasing temperature was supported by muscle kinematics and power output. At warmer (15 and 20°C) temperatures cunner pectoral fin muscles were capable of maintaining high power output at high frequencies. Low power output and locomotor capabilities were observed at $\leq 10^\circ\text{C}$, supporting the observation of a behavioural shift at this temperature. Observations made in this study demonstrate the physiological cost of performance through an ecologically relevant temperature regime. Additionally, this work suggests that further warming in the Northwest Atlantic will affect their physiological cost of overwintering. Similar experiments will be replicated on a closely related, commercially important, species (tautog) which migrates once water temperatures drop below 10°C .

A9 INTEGRATIVE MODELLING APPROACHES TO THE FISH CARDIO-RESPIRATORY SYSTEM UNDER ENVIRONMENTAL CHANGE - IS IT TIME FOR A FISH PHYSIOME INITIATIVE?

ORGANISED BY: MICHAEL BERENBRINK (UNIVERSITY OF LIVERPOOL, UK)
AND GINA GALLI (UNIVERSITY OF MANCHESTER, UK)

A9.1 MULTISCALE SYSTEMS BIOLOGY AND THE PHYSIOME PROJECT

📅 THURSDAY 6 JULY, 2017 ⌚ 09:00

👤 PETER J HUNTER (UNIVERSITY OF AUCKLAND, NEW ZEALAND)

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Multi-scale computational models of organs and organ systems are being developed under the umbrella of the Physiome Project of the International Union of Physiological Sciences (IUPS) and the Virtual Physiological Human (VPH) project funded by the European Commission. These computational physiology models deal with multiple physical processes (coupled tissue mechanics, electrical activity, fluid flow, etc) and multiple spatial and temporal scales. They are intended both to help understand physiological function and to provide a basis for diagnosing and treating pathologies in a clinical setting. A long term goal of the project is to use computational modeling to analyze integrative biological function in terms of underlying structure and molecular mechanisms. It is also establishing web-accessible physiological databases dealing with model-related data at the cell, tissue, organ and organ system levels. The talk will discuss the current state of the standards, databases and software being developed to support robust and reproducible multiscale systems biology models for the VPH/Physiome project and the possibilities for using this framework for a 'Fish Physiome'.

A9.2 MODELLING GAS EXCHANGE IN THE FISH GILL

📅 THURSDAY 6 JULY, 2017 ⌚ 09:40

👤 HANS MALTE (AARHUS UNIVERSITY, DENMARK)

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Any gas exchanger is characterized by two efficiencies. One measures how close the respiratory medium (water or air) equilibrates with the venous blood, while the other expresses how closely blood equilibrates with the inspired medium. The fish gill, by virtue of its counter-current arrangement of water and blood flows, has the potential to exchange gases with the highest possible efficiency. Thus, in an ideal gill, with perfect matching of blood and water flow, it is possible to fully equilibrate expired water with venous blood at the same time as arterial blood fully equilibrates with inspired water. In idealized gas exchangers, with linear blood gas equilibrium curves, the efficiencies are determined by two conductance ratios, the diffusion to perfusion conductance ratio (G_d/G_p) and the ventilation to perfusion conductance ratio (G_v/G_p). Perfect gas exchange in the fish gill occurs when $G_v/G_p = 1$ and G_d/G_p approaches infinity. However, a number of factors disturb this ideal situation. For example, diffusion conductance is not infinite, blood and water flows are unequally distributed to the functional gill units, and the pulsatile water and blood flows are not necessarily in phase. I will discuss how gas exchange in the gill is affected by these imperfections. I will also discuss the influence of the nonlinear blood gas equilibrium curves, and show how any adjustments of branchial O_2 uptake inevitably affects acid-base status of the blood.

A9.3 MODELLING MAXIMAL OXYGEN CONSUMPTION RATES IN FISHES

THURSDAY 6 JULY, 2017 09:55

TOBIAS WANG (AARHUS UNIVERSITY, DENMARK),
HANS MALTE (AARHUS UNIVERSITY, DENMARK)

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The ability of the cardiorespiratory system to transport oxygen is the major determinant for the rate of oxidative phosphorylation in vivo and systemic oxygen delivery therefore determines the extent to which fish and other animals can endure locomotion and digestion by means of sustainable aerobic metabolism. Limitations to systemic oxygen delivery are typically studied in isolation addressing the ability of a single organ. The convective and diffusive steps of the oxygen transport cascade, however, are arranged in series, which implies mutual dependency of each individual step where limitations or capacities at one step will influence the mass transfer of oxygen at the subsequent steps. The mutual interdependencies can be understood by using integrative mathematical models to predict the maximal rate of oxygen consumption at given values for oxygen diffusion in gills and tissue, blood oxygen binding characteristic and blood flows. When such an approach is based on physiologically realistic input parameters, it is possible to evaluate the functional significance of altering a single step in the oxygen transport cascade, such as gill diffusing capacity, cardiac output or blood oxygen carrying capacity. Not surprisingly, this approach reveals numerous limitations within the oxygen transport cascade and we will use our models to highlight the physiological processes that require additional studies and we will discuss the limitations of the theoretical approach. In particular, we will encourage that the next series of models incorporate a better appreciation of the regulation of arterial blood gases and blood pressure by means of chemo- and baroreceptors, respectively.

A9.4 HEART PERFORMANCE DETERMINATION IN LARVAL FISH USING HEART SHAPE AND VOLUME MODELING

THURSDAY 6 JULY, 2017 10:10

WARREN W BURGGREN (UNIVERSITY OF NORTH TEXAS, UNITED STATES), PRESCILLA PERRICHON (UNIVERSITY OF NORTH TEXAS, UNITED STATES), MARTIN GROSELL (MIAMI UNIVERSITY, UNITED STATES)

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Understanding cardiac function in developing larval fish is crucial for assessing physiological condition. Cardiac output measurements in transparent fish larvae and other vertebrates have long been made by analyzing videos of the beating heart, and then modeling this structure using a conventional simple prolate spheroid shape model. Larval fish hearts change shape during early development and subsequent maturation, but no studies have considered the effect of different heart geometries on cardiac output estimation. We assessed the validity of three different heart models (prolate spheroid, cylinder, cone tip+cylinder) applied to digital images of cardiac cycles, including both diastolic and systolic contraction events in larval mahi-mahi (*Coryphaena hippurus*) and red drum

(*Sciaenops ocellatus*). The inherent error of each model was determined to allow for more precise calculation of accurate stroke volume and cardiac output. The conventional prolate spheroid and cone tip+cylinder models yielded significantly different stroke volume values from 56 to 108 hours post fertilization in both red drum and mahi. End-diastolic and stroke volumes modeled by just a simple cylinder shape were 30-50% higher compared to the conventional prolate spheroid. However, when these values of stroke volume were combined with heart rate to calculate cardiac output, no significant differences between models emerged because of inherent heart rate variability. Essentially, the conventional prolate spheroid shape model provides a precise measurement of stroke volume and cardiac output. However, assessment of heart function should consider larval heart shape on a species-by-species and developmental stage-by-stage basis for most accurate calculations of cardiac output.

A9.5 FISH PHYSIOLOGY, BEHAVIOUR AND ECOLOGY UNDER ENVIRONMENTAL CHALLENGES

THURSDAY 6 JULY, 2017 10:55

PAOLO DOMENICI (CNR-IAMC, ITALY)

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Effective conservation management requires models that make projections beyond the range of available data. One way to deal with such an extrapolation is to use a mechanistic approach based on physiological processes underlying climate change effects on organisms. Using examples based on habitat selection of a coastal fish species, and on the potential competition between an invasive and a native species, here I illustrate a simple model based on aerobic scope integrated with oceanographic data of current and future scenarios, to characterize fish habitat suitability. A further step to improve our ability to predict habitat selection in fish, is to incorporate behaviour in our modelling approach, since animal behaviour can be regarded as the expression of what animals do within constraints imposed by physiology. Here, we present an example of modelling habitat choice in hypoxic conditions, based on fish physiology and behaviour. The model considers explicit and coupled energy and oxygen budgets with a diel and vertical representation of encounter processes for a planktivorous fish with both its piscivorous predators and zooplankton prey. We investigated the emerging trade-offs as individuals must balance oxygen flow, energy flow, predation risk, and starvation risk in a vertically-structured water column. We show how hypoxic layers may decrease mortality by serving as a refuge from predators. Finally, I will briefly discuss the inclusion of behaviour and physiology in ecological models as an important step to increase our ability to predict how inter-specific interactions modulate the effects of climate change on the distribution of organisms.

A9.6 EARLY EXPOSURE TO CHRONIC HYPOXIA INDUCES SHORT AND LONG-TERM REGULATION OF HEMOGLOBIN GENE EXPRESSION IN EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*)

THURSDAY 6 JULY, 2017 11:25

LAURA CADIZ (IFREMER, FRANCE), ARIANNA SERVILI (IFREMER, FRANCE), PATRICK QUAZUGUEL (IFREMER, FRANCE), LAURIANE MADEC (IFREMER, FRANCE), JOSÉ LUIS ZAMBONINO-INFANTE (IFREMER, FRANCE), DAVID MAZURAI (IFREMER, FRANCE)

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European sea bass (*Dicentrarchus labrax*) inhabits coastal waters and may be exposed to hypoxia at different life stages, requiring physiological and behavioral adaptation. In the present study, we attempted to determine whether regulation of hemoglobin (Hb) gene expression plays a role in the physiological response to chronic moderate hypoxia in whole larvae and hematopoietic tissues (head kidney and spleen) of juveniles. We also tested the hypothesis that hypoxia exposure at the larval stage could induce a long-term effect on the regulation of Hb gene expression. For this purpose, *D. labrax* were exposed to a non-lethal hypoxic condition (40% air saturation) at the larval stage from 28 to 50 days post hatching (dph) and/or at the juvenile stage from 196 to 296 dph. Data obtained on larvae indicate that hypoxia induced a subtype-specific regulation of Hb gene expression, with significant decrease of MN-Hba3, MN-Hbb4 and MN-Hbb5 and increase of MN-Hba2, LA-Hba1 and LA-Hbb1 transcript levels. Hypoxia did not induce regulation of Hb gene expression in juveniles, except in the head kidney for those that experienced hypoxia at the larval stage. The latter exhibited a significant hypoxia-induced stimulation of MN-Hba2, LA-Hba1 and LA-Hbb1 gene expression, associated with stimulation of the PHD-3 gene involved in the hypoxia-inducible factor oxygen-sensing pathway. We conclude that subtype- and stage-specific regulation of Hb gene expression plays a role in the physiological response of *D. labrax* to cope with hypoxia and that early exposure to low oxygen concentration has a long-term effect on this response.

A9.7 CAPACITIES AND LIMITS TO CONVECTIONAL RESPIRATORY GAS TRANSPORT IN FISHES

THURSDAY 6 JULY, 2017 13:50

TONY FARRELL (UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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Delivering oxygen from the surrounding water to the mitochondria in cells is critical to sustaining life, as well as achieving lifetime fitness. But are we ready for a physiome initiative for fishes? If we are to develop a reliable and informative quantitative description of physiological dynamics and functional behaviour of an intact organism, that is a physiome, first we must reach clear agreements as to what rules govern the dynamics of the oxygen cascade at each hierarchical level and what strategies generate functional behaviours. This talk will illustrate some of the fixed rules and flexible strategies that I see as being important from the perspective of cardiac physiology, which is only one of the convection steps that affects whole animal respiration (oxygen uptake), i.e., arterial oxygen transport. As examples, I will use fish responses to hypoxia and temperature, as well as intraspecific comparisons. In terms of cardiac physiology, the understanding of the critical hierarchical linkages among organ-level, cellular-level and genomic-level strategies are only in their infancy. While the need to establish a firm definitional foundation may seem like a step backwards to achieve the required leap forward, it is an important step because, if ignored, it could lead to erroneous conclusions when interpreting data, which is another point that will be emphasized.

Funded by NSERC Canada.

A9.8 PROS AND CONS OF THE CARDIORESPIRATORY SYSTEM IN SOCKEYE SALMON AS A MODEL

📅 THURSDAY 6 JULY, 2017 ⌚ 14:30

👤 ERIKA J ELIASON (UNIVERSITY OF CALIFORNIA SANTA BARBARA, UNITED STATES)

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Over the last 70 years, sockeye salmon have extensively been used as a model system for studying the cardiorespiratory system of teleost fishes. Pioneering studies by Brett in the 1950s, 60s and 70s examined how metabolic rates vary with body size, temperature, and swimming speed in sockeye salmon. More recent studies suggest that genetically distinct populations of sockeye salmon are locally adapted to their specific migration conditions. Specifically, populations with more challenging migrations have greater swim performance, higher aerobic scope, elevated cardiac scope, and larger hearts with more compact myocardium. Numerous ecological studies have used acoustic and radiotelemetry techniques to link spawning migration failure with elevated river temperatures. Some of this mortality may be mechanistically attributed to insufficient aerobic scope at elevated temperatures. Current research examining the mechanisms of thermal tolerance suggests that the heart plays a role in determining thermal tolerance in sockeye salmon and enhanced cardiomyocyte Ca^{2+} cycling may augment cardiac capacity at high temperature. The pros and cons of using sockeye salmon as a model system for a fish physiome initiative will be discussed.

A9.9 AUTONOMIC REGULATION FACILITATES ACUTE THERMAL TOLERANCE IN RAINBOW TROUT: WHOLE ANIMAL AND PERFUSED HEART PERSPECTIVES

📅 THURSDAY 6 JULY, 2017 ⌚ 14:45

👤 MATTHEW J H GILBERT (DEPARTMENT OF ZOOLOGY UNIVERSITY OF BRITISH COLUMBIA, CANADA), VARSHA RANI (LAND AND FOOD SYSTEMS UNIVERSITY OF BRITISH COLUMBIA, CANADA), SEAN MCKENZIE (DEPARTMENT OF ZOOLOGY UNIVERSITY OF BRITISH COLUMBIA, CANADA), ANTHONY P FARRELL (LAND AND FOOD SYSTEMS AND DEPARTMENT OF ZOOLOGY UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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In ectotherms, cardiac frequency and output usually increase with temperature and reach a peak before declining. A reasonable interpretation of these observations is that systemic oxygen delivery becomes limited at high temperatures, especially if the heart becomes arrhythmic, and consequently may contribute to setting upper thermal tolerances. Here we determined the extent to which rainbow trout use autonomic regulation of heart rate near their critical thermal maximum (CT_{max}) and the importance of this autonomic regulation in setting CT_{max} . Rainbow trout were fitted with ECG electrodes, cannulated to inject cholinergic and adrenergic blockers and acutely warmed in a respirometer until they lost equilibrium (CT_{max}). Fish had both cholinergic and adrenergic tone over the entire range of tested temperatures,

but adrenergic tone decreased near CT_{max} . Autonomic blockade significantly reduced CT_{max} by up to 3°C when blockers were injected individually or together. To confirm direct autonomic effects on the heart, the in-situ, working, perfused heart was exposed to the same acute warming challenge up to the temperature that the heart first developed arrhythmia. The heart was then cooled to recover rhythmicity and the warming protocol was continued with adrenergic stimulation, which produced dose-dependent increases in both the temperature and heart rate at which arrhythmia first started. Together, our results suggest that fish employ adrenergic stimulation and cholinergic inhibition of the heart to achieve CT_{max} . Supported by NSERC Canada

A9.10 TEMPERATURE INDUCED CARDIAC REMODELING IN FISH

📅 THURSDAY 6 JULY, 2017 ⌚ 15:00

👤 TODD E GILLIS (UNIVERSITY OF GUELPH, CANADA)

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The heart of some fish demonstrates remarkable phenotypic plasticity, changing form and function in response to a change in physiological condition. Through a series of studies we have demonstrated that thermal acclimation of trout alters the Ca^{2+} sensitivity of the cardiac myofilament as well as the magnitude and rate of pressure generation by the intact ventricle. Thermal acclimation of trout and zebrafish also results in significant changes to the morphology and composition of the heart. For example we, and others, have demonstrated that cold acclimation of trout causes cardiac hypertrophy and increases the connective tissue content of the heart, while warm acclimation decreases cardiac connective tissue and causes atrophy of the myocardium. In contrast, cold acclimation of zebrafish decreases the amount of thick collagen fibers in the heart. The connection between thermal acclimation and cardiac remodeling in fish has not been identified. One potential link is a change in blood viscosity altering the hemodynamic load on the heart. Such a change would put additional biomechanical strain on the heart, a condition that causes an increase in the production of TGF- β 1 by the mammalian myocardium. We have recently demonstrated that TGF- β 1 increases collagen deposition by cultured trout cardiac fibroblasts and initiates changes in the expression of gene transcripts for matrix metalloproteinases (MMPs), tissue inhibitors of MMPs and collagen isoforms, similar to that caused by cold acclimation of the trout heart. These results suggest that TGF- β 1 may be a molecular trigger that initiates temperature-induced changes in the composition of the trout heart.

A9.11 CARDIORESPIRATORY THERMAL TOLERANCE IN MARINE ECTOTHERMS AND THE EFFECT OF HYPOXIA ON THEIR UPPER THERMAL NICHE BOUNDARIES

📅 THURSDAY 6 JULY, 2017 ⌚ 15:45

👤 RASMUS ERN (UNIVERSITY OF TEXAS AT AUSTIN, UNITED STATES), ANDREW J ESBAUGH (UNIVERSITY OF TEXAS AT AUSTIN, UNITED STATES)

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Marine ecosystems are facing a rise in the frequency and severity of transient heat waves and aquatic hypoxia. The critical thermal maximum (CT_{max}) defines the upper boundary of a species' fundamental thermal niche. Marine ectotherms largely occupy the extent of latitudes tolerable within their thermal niche boundaries. Following the 'oxygen limitation hypothesis', CT_{max} of marine ectotherms decline with declining water oxygen tension ($P_w O_2$) because CT_{max} is caused by a temperature-induced collapse of the cardiorespiratory system. Aquatic hypoxia is therefore projected to impact marine ecosystems by reducing latitudinal distribution ranges and resilience to transient heat waves across species. However, the 'oxygen limitation hypothesis' was recently proven not universally applicable to marine ectotherms. Knowledge on the extent to which hypoxia reduces the CT_{max} of species from different marine ecosystems is therefore essential to forecasts of climate-induced distribution changes. The oxygen limit for thermal tolerance (PCT_{max}) is the $P_w O_2$ where an organism's CT_{max} starts to decline. The PCT_{max} can be used to assess the thermal tolerance of the cardiorespiratory system, and determine the effects of hypoxia on upper thermal niche boundaries. We determined PCT_{max} in 8 tropical, temperate, and polar species. The thermal tolerance of the cardiorespiratory system (determined via PCT_{max}) increased with habitat temperature. Only Antarctic krill conformed to the 'oxygen limitation hypothesis'. Interestingly, the 'oxygen limitation hypothesis' was founded primarily on data from polar, stenothermal species. We conclude that aquatic hypoxia is unlikely to impact the distribution of tropical and temperate species via direct limitations on the upper thermal niche boundaries.

A9.12 THE STRUCTURAL AND FUNCTIONAL FACTORS DETERMINING VO_2 MAX IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AND YELLOWFIN TUNA (*THUNNUS ALBACARES*)

📅 THURSDAY 6 JULY, 2017 ⌚ 16:00

👤 PHILLIP R MORRISON (UNIVERSITY OF BRITISH COLUMBIA, CANADA), COLIN J BRAUNER (UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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Increased O_2 uptake, transport, and delivery through the O_2 transport cascade represent major physiological adaptation to increase maximal aerobically sustained exercise. In fishes, the cardiovascular system is proposed to be the predominant rate-limiting step to O_2 delivery at VO_2 max. However, the fish O_2 transport cascade has not been modeled as an integrated system to assess the relative contribution of each component part to VO_2 max. To this end we used a steady-state model of O_2 transport that includes four conductance steps - gill ventilation, gill diffusion, cardiovascular perfusion, and tissue diffusion - and empirical data from two published studies on rainbow trout and one on yellowfin tuna. The importance of the Bohr effect to haemoglobin- O_2 offloading was assessed, and the relative contribution of each conductance step to the overall sensitivity of O_2 flux was calculated as the fractional change in VO_2 max caused by a 5% change in each step. Including a Bohr effect in the tissues was necessary for *in silico* VO_2 max values to match published values. Thus, the large Bohr effect that is typical of most teleost haemoglobins likely benefits tissue- O_2 uptake during exercise. Cardiovascular perfusion and tissue diffusion are the major limiting factors to VO_2 max in tuna and one trout model, although gill diffusion was most important for the second trout model. These apparent diffusion limitations, however, do not appear to result from structural limitations to diffusion. Thus, the greatest limitation to VO_2 max was observed at the level of the cardiovascular system (i.e., cardiac output, and haemoglobin- O_2 binding characteristics).

A9.13 MICROVASCULAR ADAPTABILITY TO ENVIRONMENTAL CHALLENGES AND THE MODELLING OF PERIPHERAL OXYGEN DELIVERY TO SKELETAL MUSCLE

THURSDAY 6 JULY, 2017 16:15

STUART EGGINTON (UNIVERSITY OF LEEDS, UNITED KINGDOM)

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Analysis of complex physiological systems requires knowledge about function and structure (encompassing genomic and proteomic information). Constructing a module of the physiome describing peripheral oxygen supply and demand at first appears quite straightforward: quantify cardiovascular transport of O_2 by convection, microvascular capacity for diffusive delivery of O_2 , influence of tissue composition on exchange, and O_2 utilisation by mitochondrial enzymes. Some basic principles become evident, e.g. allometric scaling. Anatomical and functional separation of oxidative and glycolytic muscle systems within the fish trunk simplifies handling of differential responsiveness of fibre types (cf mixed muscles within the human physiome). The impressive capacity for skeletal muscle remodelling when physiologically challenged provides an excellent test bed for any hypothesis-generating computational model.

Recognising the influence of environmental factors will be key to the success of a fish physiome initiative. For example, related species within a given thermal environment may have different convective delivery according to the ecological niche they inhabit. Whereas most temperate species display a good correlation between capillary supply and fibre mitochondrial content, those inhabiting extremely cold water appear to be outliers. The benefits of an integrative modelling approach is evident when contrasting species-specific adaptive strategies, e.g. hypoxia induces a dramatic angiogenesis in tench, a modest response in catfish, and capillary rarefaction in carp skeletal muscle. Conflicting influences can modify established relationships, e.g. cold-induced fibre hypertrophy induces a more powerful angiogenic response than seen during muscle development, such that effective capillary density is much less sensitive to changes in fibre size.

A9.14 THE ROLE OF MITOCHONDRIA IN HYPERTHERMIC DEATH

THURSDAY 6 JULY, 2017 16:30

ANTHONY (TONY) JR HICKEY (AUCKLAND UNIVERSITY, NEW ZEALAND)

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Aerobic scope of ectothermic animals has been central to climate change studies predicting thermal limits on species distributions. The limits on, and collapse of aerobic scope (AS) at high temperatures has been used to model aquatic species limits in warming ecosystems. While oxygen availability at high temperatures has been proposed to limit AS in the Oxygen Capacity Limited Thermal Tolerance (OCLTT) model, this has received criticism. For terrestrial animals oxygen is abundant, yet they still have clear thermal limits. The World's greatest marine diversity is also centred in the tropics, and while oxygen solubility declines with temperature, oxygen diffusion rates increase. Moreover, the OCLTT model appears to be inconsistent across ecotypes. Although the explanatory role of oxygen in the OCLTT may not be clear, AS is thermally limited and indicates an apparent limit on metabolism.

The currency of life is ATP. ATP is formed by processes of substrate level, and oxidative phosphorylation (OXPHOS). Only OXPHOS can sustain independent animal life, this is performed by once parasitic and now endosymbiotic mitochondria, and these provide the bulk of a cell's routine and recovery ATP. Temperature impacts OXPHOS efficiencies independent of oxygen concentration, such that thermally stressed mitochondria make insufficient ATP. Mitochondria are also not solely ATP sources. They play vital cell signalling roles and instigate apoptotic and necrotic death pathways. Overall OXPHOS inefficiencies under chronic hyperthermic stress, may starve energy hungry pathways such as growth, and at acute extreme temperatures mitochondria can even parasitize ATP, and this may mediate hyperthermic death.

A9.15 INTEGRATIVE MODELLING APPROACHES TO THE FISH CARDIO-RESPIRATORY SYSTEM UNDER ENVIRONMENTAL CHANGE - IS IT TIME FOR A FISH PHYSIOME INITIATIVE?

THURSDAY 6 JULY, 2017 16:45

MICHAEL BERENBRINK (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM)

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Fishes constitute important model systems for studies examining the effects of climate change on our oceans and rivers. Quantitative studies of the response of the fish cardio-respiratory system to external challenges have a rich history but in the past have largely been limited to the whole organism and organ system levels and been aimed at exploring maximal capacities of mechanisms. This overview explores on the one hand to what extent these mechanisms can be integrated with mechanisms on molecular and cellular levels of biological organisation. On the other hand it will be explored how these mechanisms will be affected by environmentally relevant

challenges in terms of their plasticity and genetic variability. It is argued that in order to explore quantitative integration of those systems it is necessary to get the experts on cardiac function, blood respiratory gas transport, red blood cell and haemoglobin function, acid-base and ion regulation, gill function and tissue capillarisation, but also fish energetics, to engage with bio-mathematicians. This approach is analogous to the human physiome initiative with the difference that physiological responses are modeled as a function of environmental changes rather than disease. Thus, the question is whether the time has come for a fish physiome (here proposed to be called ichthyome or pisciome) initiative among fish physiologists/ecologists. Given the discussions about a potential limitation of fish thermal tolerance by the capacity of their oxygen transport systems, especially the cardio-respiratory system, the question is timely and invites contributions from all areas of experimental biology.

A9.16 INTEGRATIVE RESPIRATORY ASSESSMENT PARADIGM (IRAP) AS AN INDEX OF A FISH'S METABOLIC CAPACITY

WEDNESDAY 5 JULY, 2017 POSTER SESSION

YANGFAN ZHANG (THE UNIVERSITY OF BRITISH COLUMBIA, CANADA), GUY CLAIREAUX (UNIVERSITÉ DE BRETAGNE OCCIDENTALE, FRANCE), DENIS CHABOT (MAURICE LAMONTAGNE INSTITUTE FISHERIES OCEANS CANADA, CANADA), ANTHONY P. FARRELL (THE UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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Environmental effects on fish metabolism have long interested experimental biologists. Generations of physiologists have been pursuing accurate, comprehensive and high-throughput methodologies to quantify metabolic capacity, i.e., aerobic and anaerobic capabilities. Typically, either aerobic scope or hypoxia tolerance are measured, but rarely are the two traits measured together. Thus, we developed an integrative respiratory assessment paradigm (IRAP) that characterizes aerobic and hypoxic capacities in using an automated, multiple-channel (8 fish), intermittent-flow respirometry system. The automation allows measurements of oxygen uptake over a period of 4 days such that standard metabolic rate (SMR) and 9 additional metabolic indices are generated. While the methodology represents a high throughput system to assess the performance of groups of fish, inter-individual variability can also be assessed by IRAP. Indeed, some individuals are clearly more active in the respirometry system than others, and some show a circadian rhythm to their activity. Our early analysis of these activity patterns has provided new insights into the ways in which activity influences the 10 metabolic indices generated by IRAP. Thus, IRAP can characterize whole-animal metabolic phenotypes with greater detail than previous methodologies.

A9.17 CARDIOVASCULAR AND CARDIORESPIRATORY RESPONSES OF THE RED DRUM (*SCIAENOPS OCELLATUS*) TO THE COMBINE ENVIRONMENTAL STRESSORS OF HYPOXIA AND CRUDE OIL

WEDNESDAY 5 JULY, 2017 POSTER SESSION

DEREK NELSON (UNIVERSITY OF NORTH TEXAS, UNITED STATES), ANDREW J ESBAUGH (MARINE SCIENCE INSTITUTION - UNIVERSITY OF TEXAS AT AUSTIN, UNITED STATES), DANE A CROSSLEY II (UNIVERSITY OF NORTH TEXAS, UNITED STATES)

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Fish species encounter a broad spectrum of environmental stressors, both naturally occurring and those of anthropogenic origin. In the Gulf of Mexico, anthropogenic stressors can coexist potentially acting synergistically on an organism. The combined effects of hypoxia and crude oil exposure are important, relevant example of this concept, prior studies of the effect of crude oil have shown deleterious effects on multiple organisms at the level of the cardiovascular system. However questions remain regarding the combined actions of oil exposure and hypoxic stress. This study was designed to investigate how convective oxygen transport is impacted and during co-exposure to hypoxia and crude oil in the red drum (*Sciaenops ocellatus*). Our working hypothesis is that co-exposure to hypoxia and crude oil will result in greater negative effects of function that would be predicted due to a sum of these stressor's individual actions. Data to be presented will include cardiac output, arterial and venous blood gas differences, PO₂, PCO₂, pH, lactate, glucose, osmolarity and hematocrit. This study is supported by the GoMRI RECOVER Consortium to A.R. and D.C.

A9.18 MODELLING INTRACARDIAC SHUNT PATTERNS IN NON-CROCODILIAN REPTILES

WEDNESDAY 5 JULY, 2017 POSTER SESSION

LAUREN E JAMES (AARHUS UNIVERSITY, DENMARK), RENATO FILOGONIO (AARHUS UNIVERSITY, DENMARK), TOBIAS WANG (AARHUS UNIVERSITY, DENMARK)

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The undivided ventricle of the amphibian and non-crocodilian reptile heart facilitates mixing of blood from the pulmonary and systemic circulations by virtue of intracardiac shunting. Recently, a new debate concerning the underlying mechanisms dictating the direction of these shunts has emerged. One proposal states that the difference between systemic and pulmonary arterial distensibilities is the main determinant of shunt direction. It is postulated that the circuit with the greater distensibility can accommodate greater blood flow, and that blood will therefore be shunted in the direction of the more distensible circuit. The second proposal maintains a more classical explanation, arguing that autonomic regulation of vascular resistances is the major factor controlling shunt direction. This gives the pulmonary circulation a reduced conductance compared to the systemic, therefore right to left shunts, where blood bypasses the pulmonary system, dominate. To resolve these conflicting views, we

constructed a dynamic model of the heart, using the InsightMaker platform, to provide a theoretical framework of parameters that may influence cardiac shunting. Our objective is to ascertain the role of arterial distensibility in shunting, with the overall aim of identifying the major factors that influence shunt patterns. The model was built to simulate blood flow distribution under normal physiological conditions, and the distensibilities of both pulmonary and systemic arteries altered, to assess their impact on shunt direction. This modelling approach allows for all parameters to be kept constant except for the one of interest, facilitating the conclusive identification of the key factors for determining shunt direction.

A9.19 FIBRE SIZE MODULATES THE EFFECT OF TEMPERATURE ACCLIMATION ON CAPILLARY SUPPLY AND INTRACELLULAR DIFFUSION

WEDNESDAY 5 JULY, 2017 POSTER SESSION

STUART EGGINTON (UNIVERSITY OF LEEDS, UNITED KINGDOM)

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Small fish living at high and low environmental temperatures, and those acclimated in the laboratory to a 20°C difference, had a similar number of capillaries (2-3) around slow muscle fibres. The major difference among species was fibre size, which was small in goldfish resulting in a very high capillary density (around 6000 mm⁻²), but where fibre size was unaffected by temperature there was no adaptive response in the microcirculation. For example, cold and warm adapted species had a local capillary supply around twice that of cold and warm acclimated groups. Extracellular (intramuscular) diffusion distance therefore shows only weak temperature compensation. In contrast, intracellular diffusion distance is sensitive to environmental temperature, with mitochondrial separation increasing from 3µm at 30°C, as a consequence of both mitochondrial volume density and spatial organisation. The integrated response of the structural limits to aerobic capacity is calculated to maintain a high intracellular oxygen tension of 4 to 5 kPa at all temperatures.

A9.20 VARIATION IN MUSCLE FINE STRUCTURE SUPPORTS ADEQUATE PERIPHERAL OXYGEN TRANSPORT IN BOTH LOCOMOTOR AND POSTURAL MUSCLES OF NOTOTHENIROID FISHES

WEDNESDAY 5 JULY, 2017 POSTER SESSION

STUART EGGINTON (UNIVERSITY OF LEEDS, UNITED KINGDOM)

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The effect of respiratory pigments on metabolic capacity, capillary supply, and fine structure of locomotor and postural skeletal muscle from Antarctic fishes was investigated. The volume density of mitochondria in slow fibres from the locomotory pectoral muscle of the white-blooded channichthyid *Chaenocephalus aceratus* was extremely high (53%), compared with the red-blooded nototheniid *Notothenia coriiceps* (29%). This index of oxygen demand was matched by capillary supply. The postural lateral musculature

had both lower capillary supply and mitochondrial content than pectoral muscles, though the difference was much less evident in *N. coriiceps* than *C. aceratus*. Extracellular and intracellular adaptations to preserve peripheral oxygen transport were found, including greater capillary length density (tortuosity index = 1.4 vs. 1.1) and mitochondrial ribbons spanning extremely large fibres (2900 vs. 1800 µm²) in *C. aceratus*. Evidence was obtained for a lower specific oxygen consumption in icefish mitochondria (citrate synthase activity / volume of mitochondria). Modelling muscle fibre PO₂ suggests that this species has maximised the structural plasticity evident within the notothenioid fishes, such that even a modest rise in sea temperature is predicted to overwhelm its limited scope for aerobic activity.

A9.21 COMPARATIVE CONTROL OF CA²⁺ HOMEOSTASIS IN SNAPPING TURTLE AND RAINBOW TROUT CARDIOMYOCYTES SUBJECTED TO ANOXIA/REOXYGENATION

WEDNESDAY 5 JULY, 2017 POSTER SESSION

ILAN RUHR (THE UNIVERSITY OF MANCHESTER, UNITED KINGDOM), GINA GALLI (THE UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

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The hearts of freshwater turtles are exceptionally tolerant of oxygen deprivation and are capable of beating in the complete absence of oxygen (anoxia) for hours, days and even months. Despite the profound medical implications, the cellular mechanisms underlying turtle cardiac anoxia tolerance is unknown. Intracellular acidosis and Ca²⁺ overload is a major determinant of anoxic cell death in mammals. Therefore, we hypothesised that turtle cardiomyocytes would defend intracellular Ca²⁺ ([Ca²⁺]_i) during anoxia/reoxygenation, despite a profound intracellular acidosis. To this end, we subjected ventricular cardiomyocytes from the anoxia-tolerant snapping turtle (*Chelydra serpentina*) to 20 mins of simulated anoxia (zero oxygen, pH 6.8) followed by reoxygenation. For comparative purposes, we repeated this experiment in an anoxia-sensitive ectotherm, the rainbow trout (*Oncorhynchus mykiss*). Fura-2 and BCECF was used to measure [Ca²⁺]_i and pH, respectively, using epifluorescent microscopy. Anoxia decreased both the amplitude of the turtle [Ca²⁺]_i (by 58%) and contractile force, while re-oxygenation led to a partial recovery of [Ca²⁺]_i (84%) with a modest increase in contractile force. In trout cardiomyocytes, anoxia led to a smaller decrease in [Ca²⁺]_i (28%), while reoxygenation saw a dramatic increase in [Ca²⁺]_i to 155% and an increase in contractile force, often leading to cell death. Both species exhibited a reduction in pH with anoxia (7.8 to 6.8) which increased during reoxygenation to levels higher than pre-anoxia. These results suggest intracellular Ca²⁺ management may be an important aspect of anoxia tolerance in the turtle heart.

A10 BIOLOGICAL ADHESIVES: FROM BIOLOGY TO BIOMIMETICS

ORGANISED BY: JANEK VON BYERN (LUDWIG BOLTZMANN INSTITUTE FOR EXPERIMENTAL AND CLINICAL TRAUMATOLOGY, AUSTRIA) AND STANISLAV GORB (KIEL UNIVERSITY, GERMANY)

A10.1 DOUBLE NETWORKS AND SLUG GLUE: INTEGRATING MECHANICS AND SEQUENCE DATA TO CHARACTERIZE AN UNUSUALLY TOUGH HYDROGEL ADHESIVE

📅 THURSDAY 6 JULY, 2017 ⌚ 09:00

👤 ANDREW M SMITH (ITHACA COLLEGE, UNITED STATES), CASSANDRA PAPALEO (ITHACA COLLEGE, UNITED STATES), CHRISTOPHER W REID (BRYANT UNIVERSITY, UNITED STATES), JOSEPH M BLISS (WARREN ALPERT MEDICAL SCHOOL OF BROWN UNIVERSITY, UNITED STATES)

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When threatened, the terrestrial slug *Arion subfuscus* secretes a viscous material from its dorsal surface, which rapidly sets into a tough, adhesive double network hydrogel. High throughput sequencing (RNA-seq) was used to determine the primary structure of all the common proteins in the glue. All but one of the proteins are novel, but they have conserved domains with cross-linking functions. The proteins fit into two main categories. The first consists of five proteins with multiple vWFA and EGF domains. These domains are typically involved in calcium-dependent intermolecular cross-linking. The second category consists of a highly abundant and relatively diverse group of at least eleven proteins that are all relatively small (10-15 kDa), and all contain one of three known ligand-binding domains (C-lectin, C1q, H-lectin). In addition, the enzyme catalase is abundant in the glue, as is a novel protein with no known homology. These results suggest a model where calcium forms sacrificial cross-links between vWFA/EGF-rich proteins. Such cross-links would toughen the gel by stiffening it, while allowing high extensibility. The lectin-domain containing proteins are hypothesized to oligomerize to present multiple different binding sites to bring different components of the glue together. Catalase may be involved in oxidative cross-linking. Further work is needed to determine how the structure of the novel protein relates to its function; it appears to be oxidatively linked into large multimers. This work demonstrates the value of whole transcriptome sequencing in biological adhesives, which often have novel proteins.

A10.2 INNATE 'PRINTING' OF GLUE AFFECTS ROBUSTNESS OF SPIDER SILK THREAD ANCHORAGES AND HELPS TO EXPLAIN THE EVOLUTION OF AERIAL WEBS

📅 THURSDAY 6 JULY, 2017 ⌚ 09:30

👤 JONAS O WOLFF (DEPARTMENT OF BIOLOGICAL SCIENCES, MACQUARIE UNIVERSITY SYDNEY, AUSTRALIA), MARIELLA E HERBERSTEIN (DEPARTMENT OF BIOLOGICAL SCIENCES, MACQUARIE UNIVERSITY SYDNEY, AUSTRALIA)

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Building behaviour in animals extends biological functions beyond bodies. In spiders, the evolution of economic web shapes as a key for ecological success has been related to the emergence of high performance silks and thread coating glues. However, the role of thread anchorages has been widely neglected. Here we show that orb-web (Araneidae) and hunting spiders (Sparassidae) use different silk application patterns that determine the structure and robustness of the joint in silk thread anchorages. Silk anchorages of orb-webs spiders show a greater robustness against different loading situations, whereas the silk anchorages of hunting spiders have their highest pull-off resistance when loaded parallel to the substrate along the direction of dragline spinning. By using morphometric and model approaches, we show how the placement of the structural thread within the instantly produced glue patch affects pull-off forces in orthogonal loading situations. These results show that the way how glue is applied, crucially enhances the toughness of the anchorage without the need of additional material intake. Preliminary results of a broad comparative analysis of spinning patterns and structural parameters of the anchorages across the spider tree of life indicate their effective refinements throughout evolution. Our results suggest that the behavioural 'printing' of glue and silk into thread anchorages was a prerequisite for the evolution of extended silk use in a 3D-space and the emergence of superior fibres. This highlights the role of attachments in the evolution of animal architectures, and suggests a high potential of such studies to inspire novel adhesive applications.

A10.3 INVESTIGATING THE RELATIVE ROLES OF ADDUCTION AND ADHESION IN TREE FROG CLIMBING

📅 THURSDAY 6 JULY, 2017 ⌚ 09:45

👤 W JON P BARNES (UNIVERSITY OF GLASGOW, UNITED KINGDOM), IAIN D C HILL (UNIVERSITY OF GLASGOW, UNITED KINGDOM), THOMAS ENDLEIN (UNIVERSITY OF GLASGOW, UNITED KINGDOM)

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The adhesive mechanisms of climbing animals have become an important research topic in recent years because of their biomimetic implications. Here, we investigate the climbing abilities of hylid tree frogs on vertical cylinders of differing diameter and surface roughness in order to investigate the relative roles of adduction and adhesion in climbing. Tree frogs adhere using their toe pads and subarticular tubercles, mainly by wet adhesion. Our hypothesis was that, on an effectively flat surface (the largest 120 mm diameter cylinder), adhesion would be the only means by which tree frogs could avoid falling, but on the two smaller diameter cylinders (44 mm and 13 mm), frogs could additionally utilise adduction forces by gripping the cylinder either with their limbs outstretched or by grasping around the cylinder with their digits, respectively. The frogs' performance would also depend on whether the surfaces were smooth (easy for the frogs to adhere to) or rough (non-adhesive). Our findings confirmed our expectations in that frogs climbed fastest on the narrowest smooth cylinder where adduction and adhesive forces could combine, but were unable to climb the largest diameter rough cylinder. Using an optical technique to visualize substrate contact during climbing on smooth surfaces, we also observed an increasing engagement of the subarticular tubercles on the narrower cylinders. Additionally, on a multiple force plate climbing apparatus, compressive forces were recorded, particularly when climbing rough surfaces, indicating the use of a clamping grip. These results support our hypotheses and have relevance for the design of climbing robots.

A10.4 MODULAR RESILIN FUSION PROTEINS – FROM MOLECULES TO MATERIALS

📅 THURSDAY 6 JULY, 2017 ⌚ 10:00

👤 PÄIVI LAAKSONEN (AALTO UNIVERSITY, FINLAND), WENWEN FANG (AALTO UNIVERSITY, FINLAND), ALESSANDRA GRIFFO (AALTO UNIVERSITY, FINLAND), ARJA PAANANEN (VTT TECHNICAL RESEARCH CENTRE OF FINLAND LTD, FINLAND), HENDRIK HÄHL (SAARLAND UNIVERSITY, GERMANY), KARIN JACOBS (SAARLAND UNIVERSITY, GERMANY), CHRISTOPHER LANDOWSKI (VTT TECHNICAL RESEARCH CENTRE OF FINLAND LTD, FINLAND), MARKUS LINDER (AALTO UNIVERSITY, FINLAND), MERJA PENTTILÄ (VTT TECHNICAL RESEARCH CENTRE OF FINLAND LTD, FINLAND)

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We have studied the behavior of adhesive and elastic fusion proteins. The protein design included a resilin-like peptide (RLP) coupled with adhesive domains. The study presents different aspects of the proteins and materials derived from the molecular to macroscopic

scale. We employed for instance single molecule force spectroscopy, cryo-TEM tomography and quartz crystal microbalance together with spectroscopic methods to understand the assembly and behavior of the molecules and materials. The resilin behavior is much regulated by the controlled conformational changes induced by change of the pH, which are in key role in formation of adhesive materials.

A10.5 STICKING TO THE DIRTIEST SURFACES: THE MOTH-SPECIALIST SPIDER *CYRTARACHNE AKIRAI* USES PREY SCALES TO INCREASE ADHESION OF AGGREGATE SILK GLUE

📅 THURSDAY 6 JULY, 2017 ⌚ 10:15

👤 CANDIDO DIAZ (UNIVERSITY OF AKRON, UNITED STATES), AKIO TANIKAWA (UNIVERSITY OF HONGO, JAPAN), TADASHI MIYASHITA (UNIVERSITY OF HONGO, JAPAN), GAURAV AMARPURI (UNIVERSITY OF AKRON, UNITED STATES), DANIEL MAKSUTA (UNIVERSITY OF AKRON, UNITED STATES), ALI DHINOJWALA (UNIVERSITY OF AKRON, UNITED STATES), TODD BLACKLEDGE (UNIVERSITY OF AKRON, UNITED STATES)

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Contaminants decrease adhesive strength by interfering with substrate contact. Moths adhering to spider webs present an ideal model to investigate how natural adhesives overcome contamination because moth's sacrificial layer of scales rub off on sticky silk, allowing them to escape. The *Cyrtarachne* spiders evolved webs that overcome this and specialize on hunting moths. We compare the adhesive performance of *Cyrtarachne* glue to more typical spider glues to understand how *Cyrtarachne* glue overcomes dirty surfaces. We compare the spreading and adhesion of spider glues on pristine moth-wings to wings denuded of scales and smooth glass. We manipulated the hydrophobicity of these surfaces to tease apart the influence of surface chemistry vs microstructure for adhesion. High-speed videos show that upon contact with moth-wings the unusually low viscosity of *Cyrtarachne* aggregate glue allows it to seep beneath the protective scales and then to accelerate spreading along the cuticle. Other spiders' glue droplets were unable to penetrate the scales, minimizing adhesion. *Cyrtarachne* adhesion on nude moth-wings was similar to glass showing that differences in topography, not chemistry, were responsible for the increased adhesion. Making moth-wings hydrophilic increased adhesion and spreading in other species, allowing their glue to spread beneath the scales similarly to *Cyrtarachne*. Hydrophilic tests however, showed increases in bulk failure for *Cyrtarachne* leading to decreases in adhesion strength from overspreading. *Cyrtarachne* uses the extremely low viscosity of its glue to take advantage of the low surface energy and topography of moth-wings, spreading rapidly across the cuticle without sacrificing cohesive strength.

A10.6 BIOLOGICAL ADHESION OF FLATWORMS

THURSDAY 6 JULY, 2017 10:55

PETER LADURNER (UNIVERSITY OF INNSBRUCK, AUSTRIA)

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Man-made adhesives contain hazardous components which are toxic and cause skin irritations, respiratory problems or they are suspected carcinogens. Furthermore, these adhesives perform poorly in wet environments. In contrast, biological adhesives produced by animals can be considered as non-toxic, tissue compatible, and they are able to function under wet conditions. However, little is known about the mechanisms underlying biological adhesives. The free-living flatworm *Macrostomum lignano* can attach and release several times within a second on any substrate in seawater. We have identified adhesive proteins using transcriptomics, differential gene expression, Mass Spectrometry, In situ Hybridization screening, Lectin staining and pull-down, specific antibodies, and light- and electron microscopy. The flatworm duo-gland system consists of an adhesive-, and a releasing gland cell, and a modified epidermal cell, the anchor cell. We now have identified two key adhesive proteins which result in a non-adhesive phenotype upon RNAi knock-down. Flatworms comprise a diverse phylum including marine and freshwater species as well as parasitic representatives. Preliminary data suggest that adhesive proteins are not conserved between different flatworm taxa. We aim for understanding the fundamental mechanisms that mediate adhesion and release in flatworms with the goal to generate a flatworm-derived biomimetic glue that can be applied in biomedicine and industry. (supported by FWF25404)

A10.7 COMPETING WITH BARNACLE CEMENT: MICROSTRUCTURES THAT REDUCE PERMANENT UNDERWATER ADHESION OF BARNACLES

THURSDAY 6 JULY, 2017 11:25

DENNIS S PETERSEN (ZOOLOGICAL DEPARTMENT FUNCTIONAL MORPHOLOGY AND BIOMECHANICS CAU KIEL, GERMANY), STANISLAV N GORB (ZOOLOGICAL DEPARTMENT FUNCTIONAL MORPHOLOGY AND BIOMECHANICS CAU KIEL, GERMANY), LARS HEEPE (ZOOLOGICAL DEPARTMENT FUNCTIONAL MORPHOLOGY AND BIOMECHANICS CAU KIEL, GERMANY)

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Sessile marine organisms such as barnacles, mussels, and tubeworms use solidifying glues for their fixation to substrates of any kind, seemingly irrespective of their surface chemistry and topography. This implies, these glues fulfill a fundamental prerequisite for a strong and reliable adhesive joint: they readily wet all substrate materials before solidification. Therefore, to prohibit permanent adhesion of marine organisms, a surface has to prevent wetting of such glues. New developments in super-repellent surfaces have shown that so called re-entrant surface microstructures repel liquids even of extremely low surface tension independently of their inherent material's wettability. To test whether such re-entrant microstructures also reduce permanent adhesion of marine hard

foulers, the amount of biofouling, especially *Balanus improvisus*, on different silicone substrates was evaluated in a static field trial in the Baltic Sea. Silicone substrates covered with mushroom-shaped micropillars (MSMs, as re-entrant structure), i.e. micropillars with broadened terminal tips, and silicone substrates covered with simple micropillars (MPs) of the same dimension, initially accumulated similar numbers of attached barnacles. However, after 13 weeks all barnacles were detached from surfaces covered with MSMs. Instead, after 17 weeks still 42% of initially attached barnacles remained attached on the surfaces covered with MPs. Visualizations of the contact interface between surface microstructures and barnacles revealed that their cement only wet the terminal contact elements of MSMs, but completely surrounded MPs. Therefore, barnacle cement generated a much higher contact area and interlocking with MPs compared to MSMs. These findings may be of importance for future non-toxic antifouling strategies.

A10.8 ADHESION WITH APPLICATIONS TO BIOLOGICAL SYSTEMS

THURSDAY 6 JULY, 2017 13:50

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How can a lizard adhere and move on a stone wall, or a fly on a glass window, or a tree frog on a plant leaf? In this presentation I will talk on some fundamentals of adhesion with applications to bioadhesion. I will discuss how surface roughness, viscoelasticity and capillary bridges influence adhesion and adhesion hysteresis. I will present the experimental results for the adhesion between silicone elastomer and smooth and rough glass surfaces in dry and wet conditions. I will also remark on the relation between adhesion and friction.

A10.9 BIOMIMETIC ADHESIVE PROTEINS INSPIRED ON SEA URCHIN ADHESIVES

📅 THURSDAY 6 JULY, 2017 ⌚ 14:20

👤 ROMANA SANTOS (CENTRO DE CIÊNCIAS DO MAR E DO AMBIENTE FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DE LISBOA, PORTUGAL), GONÇALO DA COSTA (CENTRO DE QUÍMICA E BIOQUÍMICA FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DE LISBOA, PORTUGAL), DUARTE TOUBARRO (CENTRO DE BIOTECNOLOGIA DOS AÇORES DEPARTAMENTO DE BIOLOGIA UNIVERSIDADE DOS AÇORES, PORTUGAL), JOAQUIM MARQUÊS (CENTRO DE QUÍMICA E BIOQUÍMICA FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DE LISBOA, PORTUGAL), ANA VIANA (CENTRO DE QUÍMICA E BIOQUÍMICA FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DE LISBOA, PORTUGAL), CARLOS CORDEIRO (CENTRO DE QUÍMICA E BIOQUÍMICA FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DE LISBOA, PORTUGAL)

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In nature, marine adhesive proteins show remarkable adhesive properties, biocompatibility, and biodegradability, being considered promising candidates for the development of new environmentally friendly adhesives for biomedical and industrial applications, especially in aqueous conditions.

Chemical attachment is the main adhesive strategy of sea urchins that rely on specialized adhesive organs - tube feet. The later enclose a duo-gland adhesive system, capable of producing separately adhesive and de-adhesive secretions.

Recently, the differential proteome analysis of *Paracentrotus lividus* tube feet and of its secreted adhesive, provided an unprecedented insight into the key proteins involved in sea urchin reversible adhesion and highlighted *Nectin* as the first known sea urchin tube foot adhesive protein. This protein is highly over-expressed in the adhesive disc relatively to the motile stem, being an actual component of the secreted adhesive.

Sea urchin *Nectin* contains 6 F5/8 type C-domains, also found in sea star footprint protein Sfp-1, providing it with the ability to bind carbohydrates, also present in reversible adhesives, thus indicating a cohesive role. *Nectin* has also been shown to have an adhesive role in sea urchin embryos, being involved in substrate adhesion of embryonic cells.

Employing a biomimetic strategy seeking to identify and replicate adaptive biological attributes with potential technological applications, *Nectin* recombinant expression is in progress for the full-protein sequence, but also for some protein-fragments containing only few domains. The adhesive properties of the obtained purified recombinant proteins are being investigated using atomic force microscopy, ellipsometry and surface plasmon resonance.

A10.10 CHARACTERISING AND QUANTIFYING THE ADHESION-RELATED BEHAVIOURS OF BARNACLE LARVAE

📅 THURSDAY 6 JULY, 2017 ⌚ 14:35

👤 NICK ALDRED (NEWCASTLE UNIVERSITY, UNITED KINGDOM)

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I will begin this talk by presenting the main objectives of Working Group 1 of the new, bioadhesion-focussed, COST Action 15216; briefly outlining some shared challenges that we aim to address in the coming years. These include topics as diverse as characterisation of non-protein constituents of adhesives, heterologous production in host-vector systems and reconciling sequence similarity with structural and functional similarity in adhesive biomolecules. One such consideration when discussing the adhesion of higher organisms particularly, but all biological systems to some extent, is the role of behaviour during attachment. It is tempting to reduce the study of biological adhesion to an exercise in molecular biology/chemistry, however neglecting the importance of adhesion behaviour is risky. Body movements can be crucial in the effective formation and release of an adhesive bond and selection of surfaces for attachment is central to applied adhesion problems like biofouling. Barnacle larvae have a supremely adapted and highly complex suite of adhesion behaviours that remain poorly understood, from macro-scale surface exploration that occurs prior to settlement, down to the micro-scale movements associated with temporary adhesion. Marine biofouling is a commercially and environmentally important application for bioadhesion research, and well represented in this COST Action. Among the many biofouling organisms, barnacles are considered to be of particular commercial significance and their larval settlement is the logical point of intervention.

A10.11 TECHNICAL PATTERNING INSPIRED FROM NATURE INDUCES SCALE INVARIANT BEHAVIOURS IN WETTING AND ADHESION

📅 THURSDAY 6 JULY, 2017 ⌚ 14:50

👤 VINCENT LE HOUEROU (INSTITUT CHARLES SADRON STRASBOURG, FRANCE), VALENTIN HISLER (INSTITUT CHARLES SADRON, FRANCE), CHRISTIAN GAUTHIER (INSTITUT CHARLES SADRON, FRANCE), MICHEL NARDIN (INSTITUT DE SCIENCE DES MATÉRIAUX DE MULHOUSE, FRANCE), LAURENT VONNA (INSTITUT DE SCIENCE DES MATÉRIAUX DE MULHOUSE, FRANCE)

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Nature offers many examples of how a topographic surface pattern may control wetting or adhesion phenomena: the most popular examples are probably on one hand water-repellent properties of the lotus leaf and on the other hand the sticking capabilities of gecko. Modern techniques of texturation make possible to mimic natural surfaces in order to investigate the resulting functionality with controlled and tunable topography.

In this work, we first discuss the conditions for contact formation between soft elastic hemispheres and soft elastic substrates micropatterned with hexagonal pillars using a home-

made Johnson-Kendall-Roberts (JKR) apparatus. Both deformable solids are made of cross-linked commercial polydimethylsiloxane (PDMS). Then, we describe the structural role of the pillars (aspect ratio) in the contact hysteresis during loading and unloading. Contacts may be of various types depending on the surface microtopography, leading to very different macroscopic behaviours. We show that the affine variation of the surface pattern aspect ratio lead to a scale invariant behaviour of the contact formation.

Secondly, we discuss the wetting behaviour of these microtopographically patterned substrates. It is shown that similar wetting situations are achieved when varying simultaneously and homothetically the topographical parameters (width of the pillars and inter-pillar distance). This corresponds to the scale invariance which was previously pointed out in the adhesion experiments and this law is demonstrated for a wide range of pattern dimensions. Our results show that either of those two phenomena (adhesion and wetting) can be simply controlled by the proper choice of a dimensionless ratio of topographical length scales.

A10.22 EXPLORING THE ROLE OF MECHANICAL INTERLOCKING AND HYDRODYNAMIC FRICTION IN TREE FROG ATTACHMENT

THURSDAY 6 JULY, 2017 15:05

JULIAN K A LANGOWSKI (EXPERIMENTAL ZOOLOGY GROUP WAGENINGEN UNIVERSITY RESEARCH, NETHERLANDS), RUMMENIE ANNE (EXPERIMENTAL ZOOLOGY GROUP WAGENINGEN UNIVERSITY RESEARCH, NETHERLANDS), VAN LEEUWEN L JOHAN (EXPERIMENTAL ZOOLOGY GROUP WAGENINGEN UNIVERSITY RESEARCH, NETHERLANDS)

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Tree frogs can attach to smooth and rough surfaces using their versatile toe pads. Despite considerable progress in the understanding of the mechanisms of tree frog attachment (e.g. 'wet adhesion'), it is still not fully understood how these animals manage to attach to a wide range of natural substrates.

We present the results of an experimental investigation of the role of mechanical interlocking between superficial toe pad structures and substrate asperities in the tree frog species *Litoria caerulea* and *Hyla cinerea*. Using a rotation table setup, we quantified the adhesive and frictional contact forces and stresses of frogs clinging to smooth, nano- and microrough substrates. The transparent test-substrates enabled quantification of the contact area by frustrated total internal reflection. The maximum contact forces were not significantly different for roughnesses between 0.1 nm and 15 μm in both species. This indicates that mechanical interlocking does not contribute to attachment, assuming that a change in roughness does not adversely affect mechanical interlocking and other possibly involved mechanisms of force generation.

Further, we studied the origin of friction in whole animal attachment. The friction coefficient scales with normal load with scaling exponents of -0.81 (*L. caerulea*) and -0.86 (*H. cinerea*) indicating a strong contribution of hydrodynamic lubrication to whole animal friction independent of substrate roughness. Importantly, in friction measurements the contact area was largely formed by belly-substrate contact.

Overall, our experimental findings contribute to a better understanding of the complex interplay of attachment mechanisms in tree frogs' toe pads.

A10.13 SCALING PRINCIPLES FOR UNDERSTANDING AND EXPLOITING BIO-INSPIRED ADHESION

THURSDAY 6 JULY, 2017 15:45

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A grand challenge in the science of adhesion is the development of a general design paradigm for adhesive materials that can sustain large forces across an interface yet be detached with minimal force upon command. Essential to this challenge is the generality of achieving this performance under a wide set of external conditions and across an extensive range of forces. Nature has provided some guidance through various examples, e.g. geckos, for how to meet this challenge; however, a single solution is not evident upon initial investigation. To help provide insight into nature's ability to scale reversible adhesion and adapt to different external constraints, we have developed a general scaling theory that describes the force capacity of an adhesive interface in the context of biological locomotion. We have demonstrated that this scaling theory can be used to understand the relative performance of a wide range of organisms, including numerous gecko species and insects, as well as an extensive library of synthetic adhesive materials. We will present the development and testing of this scaling theory, and how this understanding has helped guide the development of new composite materials for high capacity adhesives. We will also demonstrate how this scaling theory has led to the development of new strategies for transfer printing and adhesive applications in manufacturing processes. Overall, the developed scaling principles provide a framework for guiding the design of bio-inspired adhesives.

A10.14 BIOADHESION OF MUCILAGINOUS SEEDS

📅 THURSDAY 6 JULY, 2017 ⌚ 16:15

👤 AGNIESZKA KREITSCHITZ (ZOOLOGICAL INSTITUTE: FUNCTIONAL MORPHOLOGY AND BIOMECHANICS KIEL UNIVERSITY, GERMANY), ALEXANDER KOVALEV (ZOOLOGICAL INSTITUTE: FUNCTIONAL MORPHOLOGY AND BIOMECHANICS KIEL UNIVERSITY, GERMANY), STANISLAV N. GORB (ZOOLOGICAL INSTITUTE: FUNCTIONAL MORPHOLOGY AND BIOMECHANICS KIEL UNIVERSITY, GERMANY)

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Seeds and fruits of many plants are able to produce a sticky, gel-like capsule, i.e. mucilage envelope, after hydration. It represents a modified cell wall and is composed of polysaccharides: pectins, hemicelluloses and cellulose. The mucilage adhesion is of importance for the seeds and fruits dispersal by the animals (egzochochory). The main mass of the mucilage envelope constitute pectins, which have a great ability to water binding. Cellulose forms characteristic 'skeleton' made of long, unbranched fibrils and prevents the mucilage release from the seed surface. The chemical composition allows distinguishing two types of mucilage in different plants. Pectic mucilage is characteristic for e.g. *Linum usitatissimum*, whereas cellulose mucilage for e.g. *Plantago lanceolata*. In our mechanical tests, both mucilage types demonstrated different adhesive properties depending on the water amount. Immediately after mucilage formation the adhesion of mucilage envelope was very low. Then it was increasing gradually with the loss of water. Maximal values of the adhesion varied between 33 N for the cellulose mucilage and 91 N for the pectic one. During further mucilage desiccation, the adhesion force was decreasing very rapidly after reaching its maximum in the case of cellulose mucilage, or decreasing gradually in the case of the pectic mucilage. Our experimental data show that different chemical composition of the mucilage influences its adhesion properties.

A10.15 BIOADHESIVE PEG-CHITOSAN NANOPARTICLES AS GENE DELIVERY VEHICLE

📅 THURSDAY 6 JULY, 2017 ⌚ 16:30

👤 SEDA KIZILEL (SEDA KIZILEL, TURKEY), UGUR BOZUYUK (KOC UNIVERSITY, TURKEY), PELIN ERKOC (KOC UNIVERSITY, TURKEY)

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Our goal here is to synthesize PEGylated adhesive chitosan nanoparticles with tumor homing peptide and use these nanoparticles as a gene delivery vehicle for glioblastoma multiforme (GBM). PEG-conjugated chitosan is essential to improve colloidal stability and water solubility of chitosan at physiological pH. Here, we selectively target amino groups of chitosan and used PEG-SVA for this conjugation reaction. We characterized reaction steps through TNBS and Ellman's assay and obtained chitosan nanoparticle diameters that ranged between 70-120 nm. These particles have the capability to pass through the tight-junctions between epithelial cells, and our future studies will focus on the gene delivery potential of these nanoparticles via nasal route. This proposed system will

have a potential to promote apoptosis and decrease viability of GBM cells. The therapeutic approach used in this project may be useful to eliminate the difficult surgical operation and heavy chemotherapies in cancer patients which negatively affect whole body and result in inefficient delivery of the drug due to the presence of blood-brain-barrier.

A10.16 LEARNING FROM NORTHERN CLINGFISH: NEW BIO-INSPIRED SUCTION CUPS ATTACH TO ROUGH SURFACES

📅 THURSDAY 6 JULY, 2017 ⌚ 16:45

👤 PETRA DITSCH (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES), ADAM SUMMERS (UNIVERSITY OF WASHINGTON, UNITED STATES)

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Normally artificial suction cups only attach to smooth surfaces. A little fish, which is suspiciously named Northern clingfish (*Gobiesox maeandricus*), can attach to a huge variety of surfaces ranging from totally smooth up to as rough as sandstone. Moreover, this little fish of the marine intertidal can even hold onto slimy biofilm covered surfaces. These abilities are highly desirable for technical applications. Previously, we showed that the suction cup's elasticity in combination with its hierarchical structures are key features enabling the fish to attach to challenging surfaces. The hierarchical structures on the disc margin consist of papillae (~150 µm) covered with rods (~5 µm), which are divided into tiny filaments at their tips (~0.2 µm). These specialized structures enable not only a perfect adaptation to the surface irregularities of a substrate, but also increase the friction properties of the disc margin. The increased friction forces act against the forces pulling the disc margin in central direction during detachment. Therefore, the increased friction properties of the disc margin delay failure of the suction cup and result in increased attachment forces. Transferring these principles, we recently developed a bioinspired suction cup. Our bioinspired suction cups gain tenacities up to 70 kPa on surfaces as rough as 0.27 mm grain size (roughest surface in the experiment). On substrates of the same roughness the bioinspired suction cups attached several weeks under water in an experimental setting. Our suction cups could be technically applied in fields such as surgery or whale tagging.

A10.17 THE ARACHNOCAMPA FISHING LINES

WEDNESDAY 5 JULY, 2017 POSTER SESSION

• JANEK VON BYERN (LUDWIG Boltzmann Institute for Experimental and Clinical Traumatology, Austria), VICTORIA DORRER (Vienna University of Technology, Austria), PETE CHANDLER (Spellbound Cave, New Zealand), INGO GRUNWALD (Fraunhofer Institute for Manufacturing Technology and Advanced Materials (IFAM), Germany), MARTINA MARCHETTI-DESCHMANN (Vienna University of Technology, Austria)

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Insects use adhesives in highly diverse ways, either for attachment, egg anchorage, mating or as active resp. passive defence. The most interesting function, however, is the use of bioadhesives to capture prey, as the bonding has to be performed within milliseconds and under unsuitable conditions (i.e. movement of prey, variable environmental conditions, unfavourable attack angle, kinetic energy of flying insects) to be nevertheless successful. While much is known about the adhesive and mechanical properties of the best example, the spider's web and its different threads, less is given for other hunters as the world-renowned glow worm *Arachnocampa luminosa*. In the following study a detailed characterization of its prey capture system from the macroscopic to the ultrastructural level is performed and its tensile and strain properties of the fishing lines measured. The results provide unique insights into the glue composition, formation and its properties, adapted to the cave habitat and prey the animals catch with their sticky fishing lines.

A10.18 INVESTIGATIONS OF ADHESION IN BIO-REPLICATED MICROSTRUCTURE SURFACES: EFFECTS OF SHAPE, SIZE, AND COMPLEXITY OF PATTERNS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

• CHARCHIT KUMAR (University of Strasbourg, France), THOMAS SPECK (University of Freiburg, Germany), HOLGER F. BOHN (University of Freiburg, Germany), VINCENT LE HOUÉROU (University of Strasbourg, France)

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Adhesion and frictional surface phenomena are widespread in biological systems as well as in most technical systems. Indeed, they are influential factors for controlling the performance and durability of many physical systems. Beside the surface chemistry, the real contact area is an important parameter to modulate adhesion and friction forces acting between soft surfaces, which decidedly depends on surface micro-structuring. Adhesion and friction also play a significant role in the interaction of biological systems. In nature, almost all the biological surfaces are organised over a diverse range of surface structuring which allows for an evolutionary optimisation of their surface functionalities which proved to be inspiring for technical products like the anti-adhesive behaviour of the leaves of the rubber tree, the self-cleaning properties of the lotus leaves, or insects trapping in carnivorous plants (slippery surfaces). Three different plant leaves were selected as biological model

surfaces, according to the different size range (0.5-100µm), distinct shape, and complexity of their surface microstructures. A two-step micro-replication technique was used to fabricate polymeric replicas directly from original plant leaves. Adhesion force measurements were performed by using a dynamic pull-off tester coupled with real-time contact recording. Adhesion force characteristics were consistently measured for each polymeric replica and for a smooth polymer surface, by detaching the contact with a model adhesive system. Results reveal that the surface micro-structuring has a significant influence on adhesion force characteristics of tested polymeric surfaces. Variations in adhesion force were observed when changing the normal applied load, and altering total contact time.

A10.20 IDENTIFICATION AND LOCALIZATION OF VARIOUS TYROSINASE ISOFORMS IN THE FOOT OF THE BLUE MUSSEL *MYTILUS EDULIS*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

• PATRICK FLAMMANG (University of Mons, Belgium), BARBARA MALDONADO (University of Liège, Belgium), ELISE HENNEBERT (University of Mons, Belgium), JÉRÔME DELROISSE (University of Mons, Belgium), CÉCILE VAN DE WEERDT (University of Liège, Belgium)

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The byssus is a proteinaceous holdfast allowing marine mussels to secure themselves on rocks in the wave-swept intertidal zone. It consists of a bunch of load-bearing threads each terminating in a flattened plaque that mediates adhesion to the substratum. Byssal threads and plaques are formed by the auto-assembly of a dozen of proteins originating from three distinct glands enclosed in the mussel foot. All these proteins differ in size, amino acid composition and sequence but they share a common distinctive feature: the presence of 3,4-dihydroxyphenylalanine (DOPA), a residue formed by the post-translational hydroxylation of tyrosine. This modified amino acid fulfils two important roles in the byssus: it mediates physicochemical interactions with the surface (adhesion) and it is involved in the formation of cross-links between the different proteins (cohesion). It is generally accepted that the latter is related to the oxidation of DOPA to DOPA-quinone. Tyrosinase, a specific enzyme co-secreted with byssal proteins, can catalyse both the o-hydroxylation of tyrosine to DOPA and the further oxidation of DOPA to o-quinone. In the present study, five tyrosinase isoforms were retrieved from a foot transcriptome of the mussel *Mytilus edulis*. They showed high similarity with tyrosinases from other mussel species and all the sequences were included in a phylogenetic analysis. The specific expression of the transcripts in the foot was experimentally confirmed by RT-PCR. Finally, *in situ* hybridization experiments demonstrated that the different isoforms are gland-specific, suggesting they might be adapted for the modification of specific proteins within the byssus.

A10.21 FROM BIVALVE CEMENT TO BIOMIMETIC MINERAL ADHESIVE

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 YAN WANG-DUFFORT (ROYAL BELGIAN INSTITUTE OF NATURAL SCIENCES, BELGIUM), THIERRY BACKELJAU (ROYAL BELGIAN INSTITUTE OF NATURAL SCIENCES, BELGIUM)

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Attachment of bivalves to a natural substrate in water is one of their physiological functions. Many types of adhesives have been inspired and developed from molecular design of these biomaterials (for instance mussel byssus). The oysters and other bivalves produce a mineral adhesive for attaching to hard surface. This adhesion is achieved by a biologically induced extraperiostracal calcification, called bivalve cement, it is very different to traditional polymer adhesive. A better understanding mechanism of the bivalve cement formation will be helpful for oyster reef building which has been considered as solution for coastal erosion, it will also lead to a medical adhesive for bone fracture healing application.

A10.23 THE IMPACT OF NAUPLIAR FEEDING LEVELS ON CYPRID ADHESIVE PRODUCTION IN THE BARNACLE *BALANUS AMPHITRITE*

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

👤 SHEELAGH CONLAN (LIVERPOOL JOHN MOORES UNIVERSITY, UNITED KINGDOM), SERINA S C LEE (NATIONAL UNIVERSITY OF SINGAPORE, SINGAPORE), SERENA L M TEO (NATIONAL UNIVERSITY OF SINGAPORE, SINGAPORE)

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B. amphitrite is a cosmopolitan barnacle that biofouls manmade structures causing large-scale costs to the maritime industry. Adult barnacles release newly hatched nauplii into the plankton to feed on phytoplankton. Nauplii moult through several stages until moulting into the specialised settlement stage, the cyprid. Settlement and adhesion of the cyprid is arguably the barnacles most important life stage as failure results in mortality. While impacts of naupliar food quality and quantity have been examined in terms of cyprid settlement little has been undertaken to examine the impact on their adhesive production. Seven different feeding regimes were used to grow newly released nauplii to cyprid stage. After storage at 6°C for 3 days 20 cyprids from each feeding regime were imaged to gain an estimate of lipid quantity. The remaining cyprids from each regime (approx. 80) were allowed to settle over 24h on acid washed glass before staining with congo red to image the cyprid adhesive. Stained adhesive plaques were photographed and the average area determined for each feeding regime. Feeding regime changes resulted in significant differences in the area of cyprid adhesive plaques. At high levels of availability, the algal species had an impact with the diatom *Skeletonema costatum* resulting in significantly smaller plaques than a mixed diet or *Tetraselmis suecica*. When fed at low levels single species diets resulted in significantly reduced sized plaques compared to mixed diets.

A10.24 LOTUS AND PITCHER PLANT: ROLE MODELS FOR SLIPPERY SURFACES IN AIR AND UNDER WATER

WEDNESDAY 5 JULY, 2017 POSTER SESSION

JUDITH L GEILS (CITY UNIVERSITY OF APPLIED SCIENCES BREMEN, GERMANY), GESA J PATZELT (FRAUNHOFER INSTITUTE FOR MANUFACTURING TECHNOLOGY AND ADVANCED MATERIALS BREMEN, GERMANY), ANTONIA B KESEL (CITY UNIVERSITY OF APPLIED SCIENCES BREMEN, GERMANY)

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Slippery surfaces can be found directly in nature but also when the principle of a natural surface is extended. The former can be observed in the carnivorous pitcher plants *Nepenthes* spp. These make use of hydrophilicity on their rim. The local surface holds a fluid film, on which insects cannot adhere and slip into the pitcher. An artificial transfer is the so-called SLIPS-approach (slippery liquid-infused porous surface), in which it is possible to create an omniphobic surface, depending on the chosen lubricant. One example of the latter is the lotus plant *Nelumbo nucifera*. The purpose of its superhydrophobicity is self-cleaning but when submerged, those surfaces hold an air layer. In a technical implementation the contained air is able to reduce the wall shear stress in flow fields. Since the water is no longer in contact with a solid anymore but next to a medium of lower viscosity, it can slip off and is less decelerated. A boehmite structure, which grows on aluminum, was used as a porous surface for both the superhydrophobic and the omniphobic surfaces. To make the boehmite structure superhydrophobic, it was silanized. The reduction of wall shear stress of the air layer could be shown in water tunnel experiments with particle image velocimetry (PIV). Following the SLIPS approach the boehmite structure was infused with different lubricants. The omniphobic performance was tested by contact angle measurements, the slip-off behavior of different liquids, calculation of the surface energy, freezing rain trials, and measurement of the adhering force of hemolymph.

A10.25 ENZYMES INVOLVED IN BIOADHESIVES PRODUCTION IN INVERTEBRATES (MUSSELS AND OYSTERS) AND MACROALGAE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

CLAIRE HELLIO (UNIVERSITÉ DE BREST, FRANCE)

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The growing demand to develop a novel, environmentally friendly antifouling or bioadhesive material is ever increasing. Bioinspiration is an attractive alternative in developing such a material, learning from nature's own designs and solutions and transferring them to solve particular problems. In order to achieve this goal, the actual mechanisms and strategies that evolution has produced need to be elucidated from the subject species. The work presented in this talk will focus on bioadhesion strategies used by marine organisms and how from fundamental studies, 1) we have developed a new bioassay for testing the activity of compounds for inhibition or promotion of adhesion of various marine organisms by studying oxidising mechanisms and key enzymatic pathways; 2) we have made major advances in the characterization of the process leading to adhesion of oysters and the adhesive composition analysis at various life stage.

A11 OPEN BIOMECHANICS

ORGANISED BY: ROB JAMES (COVENTRY UNIVERSITY, UK)

A11.1 THE MUSCLE AS A WOBBLING MASS: IMPACT RESPONSES IN A SINGLE FORMULA

📅 MONDAY 3 JULY, 2017 ⌚ 09:00

👤 MICHAEL GÜNTHER (UNIVERSITÄT STUTTGART, GERMANY), KASPER B CHRISTENSEN (UNIVERSITÄT STUTTGART, GERMANY), SYN SCHMITT (UNIVERSITÄT STUTTGART, GERMANY), TOBIAS SIEBERT (UNIVERSITÄT STUTTGART, GERMANY)

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Three years ago in a talk at the SEB meeting, one of us addressed the energetic costs of wobbling masses in human locomotion, and made an attempt to reflect on the functional implications and even potential benefits of Nature's investment in energy dissipation coming along with wobbling mass dynamics as a consequence of leg impacts on the ground. We have now made progress in determining physical properties of the main contributor to human's wobbling masses in the leg when exposed to impacts like in running and jumping: active muscle fibres. Our respective experimental design using rat muscles, first results, and conclusions are presented in a tandem talk. In this talk, we would like to focus on a more theoretical aspect: a very reduced model allowing to give a first explanation of what the main ingredients determining the dynamic response to sudden changes in length or external load are. We look, in particular, at the time scale of the response. That is, we reflect on the eigenfrequency of oscillatory muscular wobbling mass dynamics. The single formula to be examined allows to predict the time-scaling, across muscle dimensions, for oscillations occurring as responses to impacts of the muscle suspension. We show that this first check for validity gives good results when scaling from rats to humans. We also give a quick outlook where to direct attention when tracing back muscular wobbling mass properties to physiological properties.

A11.2 THE MUSCLE AS A WOBBLING MASS: IMPACT RESPONSES IN KEY EXPERIMENTS

📅 MONDAY 3 JULY, 2017 ⌚ 09:15

👤 KASPER B CHRISTENSEN (UNIVERSITÄT STUTTGART, GERMANY), MICHAEL GÜNTHER (UNIVERSITÄT STUTTGART, GERMANY), SYN SCHMITT (UNIVERSITÄT STUTTGART, GERMANY), TOBIAS SIEBERT (UNIVERSITÄT STUTTGART, GERMANY)

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As part of a tandem presentation focusing on theoretical and experimental aspects, separately, this talk will focus on the experimental part of our work, from the design development to our first results and conclusions. In terrestrial locomotion, muscles undergo damped oscillations in response to limb impacts with the ground. Muscles are also actuators generating mechanical power to allow locomotion. The corresponding elementary contractile process is the work stroke of a cross-bridge, which may possibly be disrupted by superposed oscillations. To emulate rat leg impact experimentally, we dropped fully stimulated specimens (N=9) of isolated rat (*Rattus norvegicus*, Wistar) muscle (m. gastrocnemius medialis and lateralis), clamped *ex vivo* into a custom-made C-shaped frame, on the ground. The muscle belly was patterned with sphere markers and recorded with high speed cameras. We found that shock waves induced dynamic fibre strains of approx. 0.2% in the least fatigued case. For the completely non-fatigued fibre state at $F=F_{max}$, we would predict 0.1% strain from linear extrapolation. We also calculated the stiffnesses of the whole muscle-tendon complex and the fibre material separately, as well as Young's modulus of the latter for fresh, fully active and passive fibres, respectively. Knowing these stiffnesses and the muscle mass, its eigenfrequency for responses to impacts can be quantified, which represents the time scale of muscular wobbling mass dynamics. We found eigenfrequencies of about 200 Hz for the rat gastrocnemius muscle. Because submaximal muscle force represents the ordinary locomotory condition, our results suggest that forced cross-bridge disruption is a common, physiological process.

A11.3 A FUNCTIONAL ANALYSES OF ANURAN PELVIC ANATOMY USING MUSCULOSKELETAL MODELLING OF *KASSINA MACULATA*

📅 MONDAY 3 JULY, 2017 ⌚ 09:30

👤 AMBER J COLLINGS (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), ENRICO EBERHARD (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), LAURA B PORRO (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), CHRISTOPHER T RICHARDS (ROYAL VETERINARY COLLEGE, UNITED KINGDOM)

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Generally, frogs have a derived and conserved body plan. However, pelvic morphological variation is thought to allow for different directional rotations at the ilio-sacral joint, consequently enabling locomotor diversity among species. Specifically, walking locomotion is associated with pelvic lateral rotation. Walking kinematic data from $N=4$ *Kassinamaculata* frogs suggests a mean pelvic peak-to-peak angular excursion of 11.5 degrees but how this rotation is achieved remains unclear. Which muscles contribute to this motion? How do muscle functions change with locomotor behaviour? To address these questions, we combined traditional dissection, DICE- μ CT, and digital segmentation. This anatomical data was subsequently used to build a musculoskeletal model of the hindlimb, pelvis, and spine that simulated walking with the kinematic data. Individual pelvic muscle actions were quantified by calculating muscle length change and moment arms throughout the stride cycle. The coccygeoiliacus and iliolumbaris muscles form contralateral and antagonistic pairs, acting on the ilia to produce lateral rotation of the pelvis when activated uni-laterally. Surprisingly, the muscle moment arms are time varying and coincide with the peaks and troughs in the saw-tooth patterns of muscle length change. We predict pelvic geometry may influence this relationship between muscle length and moment arm, suggesting functional specialisation with morphological variation. A comparison between walking and jumping simulation outputs will test whether pelvic morphology is functionally tuned for multiple locomotor behaviours. Finally, further analysis including the hindlimb muscles will provide a greater understanding of the functional interaction between the pelvis and hindlimb and the mechanisms driving anuran locomotion.

A11.4 WORK MINIMIZATION ACCOUNTS FOR FOOTFALL PHASING IN SLOW QUADRUPEDAL GAITS, AND PHASES USED BY PRIMATES ALLOW MORE CONTROLLED FOREFOOT PLACEMENT

📅 MONDAY 3 JULY, 2017 ⌚ 09:45

👤 JIM USHERWOOD (THE ROYAL VETERINARY COLLEGE, UNITED KINGDOM), ZOE T SELF DAVIES (THE ROYAL VETERINARY COLLEGE, UNITED KINGDOM), BENJAMIN J. H. SMITH (THE ROYAL VETERINARY COLLEGE, UNITED KINGDOM)

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Quadrupeds, like most bipeds, tend to walk with an even left/right footfall timing. However, the phasing between hind and forelimbs shows considerable variation. Here, we account for this variation by modeling and explaining the influence of hind-fore limb phasing on mechanical work requirements. These mechanics account for the different strategies used by: 1) slow animals (a group including crocodile, tortoise, hippopotamus and some babies); 2) normal medium to large mammals; and 3) (with an appropriate minus sign) sloths undertaking suspended locomotion across a range of speeds. Phases predicted to be particularly costly are not observed in nature.

While the unusual hind-fore phasing of primates does not match global work minimizing predictions, it does approach an only slightly more costly local minimum. Further, modeled instants of the 'toppling' motion in the gait cycle with normal walking phases occurs just prior to forefoot placement, which is supported with vertical force measurements of walking horses; for primate phases, these toppling instants instead occur just prior to hindfoot placement, with potential advantage in terms of controlled forefoot placement on narrow or unpredictable substrates.

A11.5 MODELLING THE IMPACT OF THE NUMBER OF WALKING LEGS ON BODY DYNAMICS AND GAIT CHOICE IN POLY-PEDAL ANIMALS

📅 MONDAY 3 JULY, 2017 ⌚ 10:00

👤 TOM WEIHMANN (UNIVERSITY OF COLOGNE, GERMANY)

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Examinations of gaits and gait-changes have been the focus of movement physiology since the very beginning of the field. While most studies focussed on bipedal and quadrupedal designs, many small species have more than four pairs of legs. Nevertheless, examinations of gait-changes in poly-pedal organisms, such as arthropods, are rare. Except for the well-known change from slow feedback controlled walking to a fast, feedforward controlled running gait, no other changes are known or are deemed to be of low significance. However, recent studies in fast moving spiders, mites and cockroaches have also revealed changed leg coordination patterns and centre of mass dynamics for the transition from intermediate to high running speeds. These changes are similar to gait transitions as found in quadrupedal vertebrates. Accordingly,

the present numerical model aims to extend available theory to poly-pedal designs and examines how the number of active walking legs affects body dynamics when combined with changing duty factors and phase relations. The study shows that higher numbers of leg pairs can prevent effective use of bouncing gaits and entailed advantages as significantly higher degrees of leg synchronisation are required. It also shows that gait changes are less apparent and tend to be overlooked since small changes in the leg coordination pattern have a much higher impact onto the COM dynamics than in locomotor systems with fewer legs. In this way, the model reveals coordinative constraints for specific gaits facilitating locomotion assessment of animals with two to many pairs of walking legs.

A11.6 WHOLE BODY DYNAMICS AND HEAD STABILISATION IN PERTURBED *ACANTHODACTYLUS BOSKIANUS* LIZARDS

MONDAY 3 JULY, 2017 10:15

JANA GOYENS (UNIVERSITY OF ANTWERP, BELGIUM),
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The vestibular system in the inner ear plays an important role in balance control by sensing linear and angular head accelerations. In running mammals and birds, the head is often found to be stabilised relative to the outside world. This not only facilitates gaze control, but also provides a stable reference frame for proprioceptive information. Running lizards undergo very large axial body undulations because of their sprawled body posture. Nevertheless, we found that *Acanthodactylus boskianus* lizards stabilise their head rotations very strongly during straight level running. In natural environments, maintaining balance is more challenging, for example because of unexpected substrate movements. In order to test whether lizards are still capable of head stabilisation under such circumstances, we applied a large sideward substrate movement to running males. The runway displaced 2.7 times the normal range of sideward movement of the body centre of mass (bCOM). In the plane of the perturbation, this caused the proximal trunk part to rotate by $5.0^\circ \pm 11^\circ$ and a lateral shift of the head by 2.8 ± 4.1 mm. Still, the lizards could confine their head rotation to less than 1° ($0.99^\circ \pm 2.25^\circ$, in opposite direction of the trunk). This could not prevent significant alterations of the bCOM path in 86% of the trials. The deviation of the bCOM was, however, much smaller than the runway displacement, and the bCOM did not consistently move in the direction of the perturbation either. It is therefore conceivable that head stabilisation assists balance control during perturbations in lizards.

A11.7 EVOLUTION AND FUNCTION OF THE DOUBLE PATELLAR SESAMOIDS IN OSTRICHES (*STRUTHIO CAMELUS*)

MONDAY 3 JULY, 2017 10:30

SOPHIE REGNAULT (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), VIVIAN ALLEN (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), JOHN R HUTCHINSON (ROYAL VETERINARY COLLEGE, UNITED KINGDOM)

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The patella (kneecap) is a sesamoid bone found within the tendon of the knee extensor muscles, and is widely present in birds. Through ancestral state reconstruction, we have previously shown that the patella evolved once in birds, its origin coinciding with the evolution of greater knee flexion and more crouched limb posture. The patella is often said to increase the mechanical advantage of knee extensor muscles by increasing their moment arms; potentially beneficial to early birds with more flexed knees. Ostriches have evolved a second patellar sesamoid more distally to the first. Whilst the proximal patella is small and occupies a similar position to the single patella of other species, the distal patella is columnar and closely attached to the tibia. We used biplanar fluoroscopy (XROMM) to capture patellar kinematics of an adult ostrich cadaver knee passively manipulated in flexion-extension. We then apply these data to a previously published ostrich musculoskeletal model to investigate the mechanics of the ostrich's double patellar sesamoids. We found both patellae to decrease muscle mechanical advantage (decreasing the tendon output force for a given muscle input force) throughout the range of knee flexion-extension, unlike other species (e.g. humans, guinea fowl) where the patella increases mechanical advantage for a period of the gait cycle. It is not clear why ostriches differ from other species studied to date, or why they possess double patellae, but we speculate that specialisation for rapid locomotion and tendon protection underlie the differences.

A11.8 DYNAMICS OF JUMPING IN KANGAROO RATS: MECHANICAL WORK AND BIARTICULARITY

MONDAY 3 JULY, 2017 10:45

MARIE J SCHWANER (UNIVERSITY OF IDAHO, UNITED STATES), DAVID C LIN (WASHINGTON STATE UNIVERSITY, UNITED STATES), CRAIG P MCGOWAN (UNIVERSITY OF IDAHO, UNITED STATES)

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Kangaroo rats are bipedal hopping rodents that use erratic, vertical jumps to evade predators (i.e., snakes and owls). Previous reported jumps can reach more than 1 metre, which is approximately 20 times hip height. Under laboratory conditions these animals jump maximally half that height. Since ankle extensors are the primary muscles involved in plantar flexion, they are likely to play an important role in propelling the animal in the vertical direction during these attempts to 'out-jump' their predators. We examined the relative contribution of mechanical work by the ankle extensors during vertical jumps of kangaroo rats (*D. deserti*). Moreover, we explored the effects of biarticularity of the distal muscle tendon

unit (MTU). We hypothesize that the amount of mechanical work done by the ankle extensors is a fixed percentage of the mechanical energy required for the jump, independent of total jump height. Since the ankle extensors of these kangaroo rats are solely biarticular, we predict that the MTU produces but also transfers energy from the larger proximal muscles. We investigated the performance of the ankle extensors and the MTU during vertical jumping by combining high speed video recordings and ground reaction forces in an inverse dynamics analysis. Data suggest that there is a linear relationship between jump height and work done by the ankle, which supports our hypothesis. This suggests that ankle extensors are required to perform more work as the jump height increases. Furthermore, data show that the biarticular MTU produces, but also transfers, mechanical work.

A11.9 TUNING THE INSTRUMENT: SPIDER INFLUENCE OVER ORB WEB VIBRATION

TUESDAY 4 JULY, 2017 13:40

BETH MORTIMER (UNIVERSITY OF BRISTOL, UNITED KINGDOM), ALEJANDRO SOLER (UNIVERSIDAD CARLOS III DE MADRID, SPAIN), CLIVE SIVIOUR (UNIVERSITY OF OXFORD, UNITED KINGDOM), FRITZ VOLLRATH (UNIVERSITY OF OXFORD, UNITED KINGDOM)

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Orb weaving spiders are prime examples of organisms that take control of their physical environment. Using up to five types of silk, they expertly engineer orb webs that function to catch prey and transmit vibrations. These multifunctional orb webs are therefore perfect models to study how organisms can influence, or even tune, physical constraints for biological functions. Here we present research investigating the extent to which vibration propagation within the orb web can be tuned. In terms of the silk material, we show that spider dragline silk is unique in its scope of vibrational, or sonic, properties. As spiders can control dragline silk sonic properties actively through their behaviour, vibration transmission in these materials is uniquely tunable. In terms of the web structure, both experimental and modelling approaches were used to quantify orb web vibration. We show that changes in web geometry, thread tensioning and silk stiffness influence all aspects of vibration propagation, including propagation speed and amplitude. Importantly, all of these factors are under active control by the spider both during and even after web building, giving spiders control mechanisms for tuning vibration propagation within the web. Overall, our research showcases the level of biological control possible over inevitable physical constraints when organisms engineer their own materials and structures.

A11.10 PRODUCTION OF ATTACHMENT SILK CARPETS IS ESSENTIAL FOR HERBIVORY IN *BICYCLUS ANYNANA* CATERPILLARS

TUESDAY 4 JULY, 2017 13:55

SIMON CHEN (DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), WALTER FEDERLE (DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), PAUL M BRAKEFIELD (DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM)

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Caterpillars must attach to the surfaces of host plants in order to feed on them. They achieve attachment not only by gripping with thoracic legs and abdominal prolegs but also by producing silk. So far, the function of caterpillar attachment devices and the role of silk for attachment are largely unexplored.

B. anynana caterpillars lay thin silk carpets onto the leaves of maize and a natural host plant, *Oplismenus compositus*, before feeding on them. To test the importance of silk, we investigated the effect of experimentally ablating the caterpillars' spinnerets on their feeding performance. For both host plants, *B. anynana* caterpillars without spinnerets generally failed to feed on upright plants, but gained weight normally when the plants were offered horizontally on the ground. Force measurements on host plant leaves and artificial substrates with and without silk carpets using a centrifuge force tester confirmed that silk results in dramatically increased attachment.

We discovered that silk-laying behaviour in *B. anynana* is tightly controlled, and occurs mainly on more slippery substrates with a smooth or micro-rough texture. Comparisons with the noctuid moths *Arctia caja*, *Callimorpha dominula*, and *Apamea unanimitis* revealed strong differences in the use of silk and in the conditions triggering the laying of silk carpets.

Our results show that silk-based attachment is an effective mechanism used by some but not all Lepidoptera. Silk allows caterpillars to achieve a firm grip on slippery plant surfaces and thereby exploit some otherwise inaccessible host plants.

A11.11 ONTOGENY, BIOMECHANICS AND DIFFERENT GROWTH HABITS OF 'FINGER-LIKE' STEM-BRANCH-ATTACHMENT REGIONS IN THE ARALIACEAE FAMILY

TUESDAY 4 JULY, 2017 14:10

KATHARINA BUNK (PLANT BIOMECHANICS GROUP BOTANIC GARDEN UNIVERSITY OF FREIBURG, GERMANY), SEMJON KRASSOVITSKI (PLANT BIOMECHANICS GROUP BOTANIC GARDEN UNIVERSITY OF FREIBURG, GERMANY), LARISSA BORN (INSTITUTE FOR TEXTILE AND FIBER TECHNOLOGIES UNIVERSITY OF STUTTGART, GERMANY), GÖTZ T. GRESSER (INSTITUTE FOR TEXTILE AND FIBER TECHNOLOGIES UNIVERSITY OF STUTTGART, GERMANY), THOMAS SPECK (PLANT BIOMECHANICS GROUP BOTANIC GARDEN UNIVERSITY OF FREIBURG, GERMANY), TOM MASSELTHER (PLANT BIOMECHANICS GROUP BOTANIC GARDEN UNIVERSITY OF FREIBURG, GERMANY)

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A 'finger-like' branching morphology with individual woody strands connecting the side branch and stem has been identified in the Araliaceae species *Schefflera arboricola*. Its functional morphology serves as concept generator for the biomimetic optimization of nodal elements in branched building constructions. Based on detailed investigations of *S. arboricola*, we studied if this conspicuous branching morphology also appears in other Araliaceae genera. We analysed how these woody strands develop and how the branching morphology changes during ontogeny, altering growth habits and biomechanical loading situations in selected Araliaceae species. A 'finger-like' branching morphology was found also in the Araliaceae species *Fatsia japonica* and *Polyscias balfouriana* yet with several differences to *S. arboricola*, concerning vascular bundle progression from the main stem into the side branches as well as ontogenetic branch development. Of particular interest is the Araliaceae species *Hedera helix*, displaying ramifications with a 'finger-like' morphology but also with partially or completely merged stem-branch-attachment regions. The different branching modes of *H. helix* are analysed for correlations with ontogeny, the altering growth habit of *Hedera* as well as changing biomechanical demands (e.g. climbing or creeping vs. self-supporting axis). Three-dimensional μ -CT models of different *H. helix* branching modes allow for the development of various braided technical ramifications consisting of fibre-reinforced composites, which are to be tested mechanically, in order to assess how different biological shapes and fibre reinforcements can be implemented in technical fibre-reinforced branchings in general and if they improve the mechanical performance.

A11.12 KNOTS AND TANGLES WEAKEN KELP FRONDS WHILE INCREASING DRAG FORCES AND HERBIVORE LOADS ON THE KELP

TUESDAY 4 JULY, 2017 14:25

NICHOLAS P BURNETT (UNIVERSITY OF CALIFORNIA - BERKELEY, UNITED STATES), MIMI A R KOEHL (UNIVERSITY OF CALIFORNIA - BERKELEY, UNITED STATES)

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The fronds of long, flexible kelp can become knotted (a single frond tied around itself) and tangled (multiple fronds intertwined) as they move back and forth with ocean waves, but the effects of knots and tangles on the assemblages of animals living on the kelp and on kelp breakage are not known. We used the kelp *Egregia menziesii* to study biomechanical and ecological consequences of knotting and tangling. Knots weakened fronds by 18% when pulled in tension, and increased hydrodynamic forces on fronds by 56%. There were more and larger herbivores on tangled fronds, which suffered greater damage by grazers than did untangled fronds. Thus, knotted and tangled fronds were more likely to break than unknotted, untangled fronds. Breakage at knots and tangles occurred most frequently in the autumn and pruned the fronds, thereby reducing the risk of the whole kelp being dislodged by large waves during winter storms.

A11.13 STRUCTURAL, MATERIAL, AND FUNCTIONAL GRADIENTS IN BIOLOGICAL ATTACHMENT SYSTEMS

TUESDAY 4 JULY, 2017 14:40

STANISLAV GORB (ZOOLOGICAL INSTITUTE KIEL UNIVERSITY, GERMANY), LARS HEEPE (ZOOLOGICAL INSTITUTE KIEL UNIVERSITY, GERMANY)

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Insects, spiders, and reptiles have developed elaborate attachment organs which allow them to attach reliably on a variety of different and unpredictable surfaces. By this combination of surface microstructures and material properties they are able to maximize real contact area and thus enhance their adhesive and frictional properties of animal feet. Whereas in smooth pads gradients of mechanical properties are well known, recent works on hairy adhesive systems in insects have revealed three important gradient-based features within individual hairs which allow for robust, reliable, and reversible adhesion. (1) The presence of a material gradient along the setae leads to an enhanced adaptability to rough surfaces and prevents clustering of setae. (2) The gradient of setal length from proximal to distal part of the pad is presumably responsible for uniform stress distribution in contact (3) The presence of joint-like elements within setae further enhances the adaptability and allows for robust adhesion. Here we will summarize structural, material, and functional gradients found in animal attachment pads. Moreover, we will present first experimental results on artificial mimics highlighting the importance of gradients for robust adhesion.

A11.14 ALLOMETRY AND THE LIZARD EAR: A MORPHOLOGICAL AND BIOMECHANICAL APPROACH

TUESDAY 4 JULY, 2017 14:55

MENELIA VASILOPOULOU-KAMPITSI (UNIVERSITY OF ANTWERP, BELGIUM), JANA GOYENS (UNIVERSITY OF ANTWERP, BELGIUM), SIMON BAECKENS (UNIVERSITY OF ANTWERP, BELGIUM), RAOUL VAN DAMME (UNIVERSITY OF ANTWERP, BELGIUM), PETER AERTS (UNIVERSITY OF ANTWERP UNIVERSITY OF GHENT, BELGIUM)

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Detection of self-motion by the vestibular system of the inner ear is crucial for maintaining balance. This system comprises 3 interconnected semi-circular canals filled with endolymph fluid. The common view is that angular head accelerations cause endolymph flow (relative to the canals), which in turn deforms sensory organs (cupulae). From a physical viewpoint, it is possible that the performance of this canal system is size-constrained: in ducts which are too narrow, viscous forces will dominate the hydrodynamics and as a result, behaviourally relevant accelerations will prevent endolymph flow. Animals that have small heads (in an evolutionary or ontogenetic context) can circumvent this problem in three ways: by having relatively larger vestibular systems (negative allometry), by altering their shape to affect the hydrodynamics, or by using locomotor behaviour that relies less on the vestibular system for balance control (e.g. lower manoeuvrability). Because the latter depends heavily on habitat type, we focussed on lacertid lizards, which have both a considerable size range and wide variety of habitats. We grouped 24 species in three habitat types and we acquired 3D models of their bony vestibular systems based on micro-CT scans. Our phylogenetic scaling analysis of linear measurements shows that smaller species have indeed disproportionately larger ears for their head size. This scaling relationship does not differ between habitats, but geometric morphometrics analyses will show whether shape adaptations exist. To conclude, the present results show that the morphology of the vestibular system in Lacertids is determined by physical size-constraints, rather than by habitat demands.

A11.15 DYNAMIC AUDITION: BIOPHYSICS OF THE TYMPANIC MEMBRANE OF THE ASIATIC WATER MONITOR LIZARD (*VARANUS SALVATOR*)

TUESDAY 4 JULY, 2017 15:10

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The water monitor (*Varanus salvator*) has internally coupled ears, an auditory system distinguished by (patent) anatomical conduits through the skull linking the middle ear cavities on both sides of the head. We describe a small skeletal muscle in *V. salvator* which inserts onto the middle ear ossicle and the tympanic membrane. A combination of laser doppler vibrometry, experimental stimulation, and pressurization of the conduits coupling the eardrums, was used to demonstrate significant changes in the vibrational velocity

and waveform pattern of the tympanic membrane. The combined anatomical and functional results suggest that *V. salvator* is capable of actively modulating the tension of the tympanic membrane. This active modulation is unlikely to have a uniform affect over the surface of the tympanum. The laser Doppler data revealed that the tympanic membrane of *V. salvator* is functionally divided into dorsal, more pliant, and ventral, stiffer, regions. The ventral portion is smaller and vibrates significantly (up to 12x) more to the same stimuli. The pattern of tympanic membrane response is highly frequency-dependent with a peak response centered around 2.5 kHz, and a second (lower) response at 0.5 kHz. This pattern of tympanic membrane frequency response coincides with the low- and high-frequency ranges of the varanid auditory response. *Varanus salvator* appears to have evolved a tympanic pressure-balance system analogous to what is known in mammals, in which one portion of the tympanic membrane preferentially responds to middle ear/Eustachian tube air pressures. Audition in these lizards is more dynamic, and complex, than previously appreciated.

A11.16 FEMORAL HEAD TRABECULAR ARCHITECTURE IN SCIUROMORPH RODENTS (*MAMMALIA*): EFFECTS OF BODY SIZE AND LOCOMOTOR TYPE

TUESDAY 4 JULY, 2017 16:00

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Trabeculae, as part of bone inner structure, build a network of thin bony struts that is constantly remodelled and therefore reflects changes in loading during life (Wolff's law). Experimental studies have shown that both the orientation and the dimensions of the trabeculae are adjusted in this context. In order to investigate how trabecular architecture reflects different locomotor types, we analysed the 3D inner bone microstructure of femoral heads in Sciuromorpha (squirrel-like rodents). This taxon is diverse in terms of body size, lifestyle and locomotor type (aerial, arboreal, fossorial). While evaluating locomotor adaptation of bone microstructure our study takes into account the effect of body size on its properties (allometric scaling). Analysis was done on high resolution computed tomography scans. A cubic volume of interest was selected in the centre of each femoral head and analysed by extraction of various parameters that characterize trabecular architecture. We show that some of these parameters (bone surface density BS/BV, connectivity density ConnD, trabecular thickness TbTh and trabecular separation TbSp) are subject to strong allometry. Degree of anisotropy DA and bone volume fraction BV/TV show weak

allometry. For all parameters except for BV/TV and trabecular main orientation, the size-corrected data show to some extent adaptive signal to the different locomotor types. In perspective, our findings can be verified in experimental studies including setups that imitate different habitats and therefore different loading patterns applied on living Sciuromorpha. This approach would further elucidate the functional significance of bone internal structure in that taxon.

A11.17 A NEW PROCEDURE OF PROCRUSTES SUPERIMPOSITION – A CASE STUDY WITH THE HUMERUS OF XENARTHANS (MAMMALIA)

TUESDAY 4 JULY, 2017 16:15

FALK MIELKE (AG MORPHOLOGIE UND FORMGESCHICHTE INSTITUT FÜR BIOLOGIE HUMBOLDT-UNIVERSITÄT ZU BERLIN, GERMANY), ELI AMSON (AG MORPHOLOGIE UND FORMGESCHICHTE INSTITUT FÜR BIOLOGIE HUMBOLDT-UNIVERSITÄT ZU BERLIN, GERMANY), ANNEKE H VAN HETEREN (SEKTION MAMMALOGIE ZOOLOGISCHE STAATSSAMMLUNG MÜNCHEN STAATLICHE NATURKUNDLICHE SAMMLUNGEN BAYERN, GERMANY), JOHN A NYAKATURA (AG MORPHOLOGIE UND FORMGESCHICHTE INSTITUT FÜR BIOLOGIE HUMBOLDT-UNIVERSITÄT ZU BERLIN, GERMANY)

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Conventional geometric morphometrics usually involves Generalized Procrustes Analysis. In this process, specimens are aligned to their mean shape (Procrustes Superimposition). The procedure treats a landmark data set as uniform, ignoring crucial biomechanical constraints that operate differentially upon the evolution of functional modules of a structure. This may be particularly problematic for limb long bones, which possess articular surfaces that differ remarkably from the rest of the bone in their mechanical adaptation. Articulations are analogous to hinges that keep the bone in relative position, whereas some other substructures may represent optimized lever arms for muscle attachment. To demonstrate the consequences of these differential biomechanical constraints for functional inference from 3D surface landmark data, we analysed the humeri of extant xenarthrans, which were digitised using micro-CT or laser surface scanning. We show that in traditional geometric morphometric methods, which consider the full bone at once, shape information conveyed by only the non-articular landmarks dominates the superimposition process and blurs the resulting shape change components. For analyses that tackle questions of biomechanics, we suggest a modification of the universally applied Procrustes algorithms that exploits the restricted evolutionary capacity of articular surfaces. By selectively superimposing the bones based on articular surface landmarks, the remaining shape information will represent actual lever relations of muscle attachments. For studies concerned with functional adaptations of the musculoskeletal system, our analysis procedure can improve shape differentiation between groups of locomotor types and lifestyles. Furthermore, our analysis may serve as a basis for biomechanical hypotheses of subsequent functional experiments.

A11.18 THE RHINOCEROS AMONG SERPENTES: COMPARATIVE ANATOMY AND EXPERIMENTAL BIOPHYSICS OF CALABARIA REINHARDTII SKIN

TUESDAY 4 JULY, 2017 16:30

DAWEI HAN (TRUMAN STATE UNIVERSITY, UNITED STATES), BRUCE A YOUNG (A.T. STILL UNIVERSITY, UNITED STATES)

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Previous studies have suggested that there are little interspecific functional or structural differences in snake skin among species. Morphological examination of the Calabar burrowing python (*Calabaria reinhardtii*) revealed a proliferation of the thickness of the integumentary layers, a highly-organized lamellate arrangement of the dermal collagen bundles, and a reduction in the size of the interscale hinge region of the integument. Comparisons with other snakes suggest that these integumentary specializations are unique to *C. reinhardtii*. Biomechanical testing demonstrated that the skin of *C. reinhardtii* is more resistant to penetration than the skin of the other tested snake species. The laminar arrangement of the collagen bundles provides for penetrative resistance, while maintaining the flexibility characteristic of snake skin. Considering the life history of this species, it is hypothesized that the specialized integument of *C. reinhardtii* is a passive defensive mechanism against penetrative bites from maternal rodents and predators.

A11.25 THREE-DIMENSIONAL MOTION ANALYSIS OF PENGUIN SWIMMING AND ESTIMATION OF THE HYDRODYNAMIC FORCE

WEDNESDAY 5 JULY, 2017 09:00

HIROTO TANAKA (TOKYO INSTITUTE OF TECHNOLOGY, JAPAN), YUSUKE IWASAKI (THE UNIVERSITY OF TOKYO, JAPAN), MASATERU MAEDA (TOKYO INSTITUTE OF TECHNOLOGY, JAPAN)

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Penguins are wing-propelled diving birds and the previous biologging studies have revealed the penguins' swimming performances such as diving depth, diving duration, and cruising speed. Their swimming mechanism, however, remains largely unknown. Particularly of interest to us is that in what manner do they flap their wings (flippers) to efficiently and effectively propel themselves and how it is related to the swimming performance. To investigate the penguin's wing kinematics and body motions, we recorded underwater swimming of a gentoo penguin, *Pygoscelis papua*, in a large water tank in an aquarium using eight waterproof video cameras at 240 frames per second. The characteristic points on the wings and body in each image frame were manually tracked, based on which the three-dimensional coordinates were reconstructed. The total force acting on the penguin was calculated by multiplying the mass by the acceleration. The body drag was estimated from a computational fluid dynamics (CFD) simulation using a simplified 3-D body model, and the buoyancy was estimated using the same body model. The hydrodynamic force was consequently obtained by subtracting the body drag and buoyancy from the total force. The preliminary results on a

slow (1 m s^{-1}) swimming case indicate that the penguin generates hydrodynamic force mainly during the upstroke phase of the wings rather than during downstroke, which is in contrast to flying birds. The angles of attack during mid-strokes were approximately 20 degrees, and the results from quasi-steady force estimation implied that the unsteady force generation mechanisms may be involved.

A11.26 SWIMMING HYDRODYNAMICS OF SYNCHRONIZATION AND COLLECTIVE SWIMMING PATTERNS IN FISH

WEDNESDAY 5 JULY, 2017 09:15

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Recent experimental evidence on tetra fish *Hemigrammus bleheri* (J.R. Soc. Interface 13:20160734) demonstrates that fish in collective swimming tend to perform synchronization, correlating with the swimming speed. As the flow rate increases to a critical level, the swimming modes are dominated by 'out-phase (half cycle phase difference)' and 'in-phase (zero phase difference)' configurations.

To elucidate the underlying mechanisms behind the highly non-linear and complicated nature of the hydrodynamics of collective swimming, we developed an integrated three-dimensional (3D) computational approach that couples the Navier-Stokes (NS) equations with the equations of undulating body motion. Kinematics of the experimental observations are applied to the model fish. To explore the benefit of schooling, we make a comparison of swimming hydrodynamics among a single fish, paired fish and a three-fish group, in terms of critical swimming speed (balance speed between thrust and drag), thrust, drag, total power, cost of transport and Froude efficiency. To unveil the mechanism of synchronization, we vary the phase shift between undulatory motion of the middle fish and its neighbors in the group of three. We test the phase differences of zero, half cycle, and one-fourth cycle relative to the fish on its left and right sides.

In the numerical simulations, we add virtual lateral lines to the model fish to record the time-dependent change of the perceived hydrodynamic pressure, to explain how fish sense and control the synchronization. Feasibility to capture and utilize the complex 3D wake structure behind the fish schooling is also discussed.

A11.27 THE PATTERN OF THRUST ON THE BODY OF A SMALL SWIMMING FISH (A HILLSTREAM LOACH)

WEDNESDAY 5 JULY, 2017 09:30

JAY WILLIS (OXFORD UNIVERSITY, UNITED KINGDOM), ADRIAN THOMAS (OXFORD UNIVERSITY, UNITED KINGDOM), THERESA BURT DE PERERA (OXFORD UNIVERSITY, UNITED KINGDOM)

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Hillstream loaches have elaborate adaptations to life in shallow fast flows. Here we present an inexpensive and quick way to visualise the pattern of thrust as a small loach (0.03m) swims and relate this precisely to its body movement and shape. We place the fish in a shallow tank. The fish swims and makes waves on the surface which, when viewed from above, cause deformations of the pattern on the base of the tank. We photograph the deformations using a standard camera and derive water velocity using Schlieren methods and standard freely available software. The surface heights can be reconstructed or pattern of thrust directly inferred from the result. The method has wide potential application and we demonstrate this using a variety of fish. The main benefits are that it is quick, inexpensive, and easy to set up. Importantly this method does not involve abnormal treatment of the fish, such as lasers, dye streams or additives to the water. Once the thrust pattern has been quantified we also have the opportunity to precisely measure the position and movement of the fish's body and link the two patterns. We wrote an application to identify the fish, its spine, and the sides of its body to draw conclusions about its swimming style. We discuss the likely impacts of the hillstream loache's highly specialised anatomy on its propulsion. We suggest several improvements to the general analysis of swimming fish derived from these experiments.

A11.28 AERODYNAMIC EFFECT OF THE DISTRIBUTED FLEXURAL STIFFNESS OF HUMMINGBIRD'S WING

WEDNESDAY 5 JULY, 2017 09:45

MASAHIRO AIZAWA (TOKYO INSTITUTE OF TECHNOLOGY, JAPAN), TAKESHI YAMASAKI (YAMASHINA INSTITUTE FOR ORNITHOLOGY, JAPAN), MASATERU MAEDA (TOKYO INSTITUTE OF TECHNOLOGY, JAPAN), HIROTO TANAKA (TOKYO INSTITUTE OF TECHNOLOGY, JAPAN)

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Hummingbird wings are composed of radially spread multiple feathers, which can lead to passive wing deformation in flapping flight due to aerodynamic and inertia forces. While the effects of the wing deformation on aerodynamics of hovering hummingbirds have been considered to be important, flexural stiffness of the feather rachis and its distribution in the wing have never been measured for the hummingbirds. In this study, we directly measured the flexural stiffness of the rachises of a museum specimen of *Amazilia amazilia*, by cantilever bending tests for multiple locations aiming to quantify the distributed flexural stiffness of the hummingbird wing. Moreover, in order to investigate

the effect of the distributed stiffness on hovering flight, we fabricated different types of wing models which were composed of a polyimide film and UV-laser-cut CFRP (carbon fiber reinforced plastic) artificial rachises mimicking the measured flexural stiffness. Each wing model was attached to an electric flapping mechanism, and the average lift and electrical power consumption were measured. The wing deformations were also three-dimensionally measured using three high-speed video cameras. The bending tests of the rachises revealed that the flexural stiffness against force application from the ventral side was twenty to hundred times larger than that from the dorsal side. Also, the result of the experiments with the wing models suggested that the hummingbird's rachises prevent excess deformation of the wing to generate sufficient lift with the minimum amount of the rachis materials.

A11.29 AERODYNAMICALLY OPTIMISED WINGBEAT KINEMATICS COMPARED TO EMPIRICAL OBSERVATIONS

WEDNESDAY 5 JULY, 2017 10:00

MARCO KLEINHEERENBRINK (UNIVERSITY OF OXFORD, UNITED KINGDOM)

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Although morphologically distinct, the independent evolution of powered flight in birds, bats and insects has resulted in functionally similar solutions: pairs of wings moving with a reciprocal motion to produce both lift and thrust. Flapping wings are an efficient solution allowing for a smooth transition between operation at low speed (helicopter) and high speed (aeroplane). Using only aerodynamic considerations, it is possible to identify energetically optimal wingbeat kinematics, such as stroke plane, wingbeat amplitude and frequency. Observed behaviour of birds recorded in literature are in line with the predictions based on the aerodynamic optimum. This underlines the importance of the aerodynamic constraints that flying animals are subjected to.

A11.30 COMPARATIVE KINEMATICS OF FLAPPING FLIGHT IN THREE DIPTERAN SPECIES

WEDNESDAY 5 JULY, 2017 10:15

INÉS L DAWSON (UNIVERSITY OF OXFORD, UNITED KINGDOM), SIMON M WALKER (UNIVERSITY OF OXFORD, UNITED KINGDOM)

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Diptera are among the most adept fliers of all insects due to their ability to quickly adjust their flight patterns in response to a changing environment. They occupy a wide range of ecological niches, which is reflected in their varying morphology and behaviour. We therefore explored how the flight of three species varied across a range of similar aerial manoeuvres. We collected free-flight kinematic datasets for *Drosophila melanogaster*, *Calliphora vicina* and *Eristalis tenax* using high-speed photogrammetry. Wingbeat kinematic parameters were then compared across the range of heading velocities used by each species. Aside from decreasing their body pitch angle with increased heading velocity, each dipteran species altered their kinematic parameters differently across their velocity range. Hovering flight was found only in *Eristalis*, whereas in *Drosophila* and *Calliphora* a low heading velocity was always accompanied with a significant vertical component, congruent with their flight behavior and biology. This was accompanied with a less variable stroke plane angle with respect to the horizontal plane in *Eristalis*, and a less variable stroke plane angle with respect to the body axis in *Calliphora*. The three species also alter their wingbeat frequency, amplitude and the timing of their wing rotation differently with increasing heading velocity. In order to further examine the aerodynamic effect of these different wing kinematic patterns, we fitted a quasi-steady blade element model to each dataset. This allowed us to compare differences in lift and drag forces across the three species to determine how they are used to control heading velocity.

A11.31 MECHANICS AND ENERGETICS OF PERCHING FLIGHT IN A STEPPE EAGLE (*AQUILA NIPALENSIS*)

WEDNESDAY 5 JULY, 2017 11:00

LYDIA A FRANCE (UNIVERSITY OF OXFORD, UNITED KINGDOM)

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Perching is a challenging behaviour for birds as it requires careful control to reach the target and sufficient braking to prevent collision. Data was taken from reconstructed points tracked using photogrammetric techniques (Carruthers et al., 2010) across the wings, tail, and body from a perching Steppe eagle (*Aquila nipalensis*). During perching, the eagle performs a shallow glide followed by a rapid pitch-up manoeuvre. This slow flight at very high angles of attack poses challenges for lift production. From these data, the dissipation of kinetic energy through aerodynamic braking is quantified. In addition, the close coordination of wing and tail morphing was measured during this manoeuvre that includes deep stall.

A11.32 TAKE-OFF DYNAMICS OF BLOOD-FED MALARIA MOSQUITOES

WEDNESDAY 5 JULY, 2017 11:15

FLORIAN T MUIJRES (WAGENINGEN UNIVERSITY, NETHERLANDS), SOFIA W CHANG (BERKELEY UNIVERSITY, UNITED STATES), WOUTER G VAN VEEN (WAGENINGEN UNIVERSITY, NETHERLANDS), MIMI A R KOEHL (BERKELEY UNIVERSITY, UNITED STATES), ROBERT DUDLEY (BERKELEY UNIVERSITY, UNITED STATES)

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Blood-fed mosquitoes can carry blood loads that are several times their own body weight. To take-off and escape from a host after blood feeding, the mosquito must thus exert forces to carry this large blood load, while at the same time it needs to avoid detection by minimizing tactile signals exerted on the host's skin. We studied this trade-off between escape speed and stealth in malaria mosquitoes, *Anopheles coluzzii*, using 3D motion analysis of high-speed stereoscopic videos of mosquito take-offs and aerodynamic modelling. We found that during the push-off phase of the escape maneuver, mosquitoes enhanced take-off speed by using wing-derived aerodynamic forces in addition to leg-based push-off forces. The wing forces contributed 61% to total push-off force, suggesting that - unlike many other animals - mosquitoes rely for a large part on aerodynamic force production at take-off. By gently extending their long legs, mosquitoes spread push-off forces over a larger time-window and thus further reduce peak leg forces. These results suggest that mosquitoes reduce tactile signals on a host by producing peak leg forces much lower than those reported for other insects; this type of take-off maneuver might be a general locomotor feature of blood-feeding insects.

A11.33 FLIGHT DYNAMICS AND BEHAVIOURS OF MALARIA MOSQUITOES AROUND ODOUR-BAITED TRAPS

WEDNESDAY 5 JULY, 2017 11:30

ANTOINE CRIBELLIER (WAGENINGEN UNIVERSITY RESEARCH, NETHERLANDS), FLORIAN MUIJRES (WAGENINGEN UNIVERSITY RESEARCH, NETHERLANDS), JOHAN VAN LEEUWEN (WAGENINGEN UNIVERSITY RESEARCH, NETHERLANDS)

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Host searching in flying mosquitoes is triggered by a species-specific combination of odours and CO₂. By using the odour blend that attracts anthropophilic malaria mosquitoes, species-specific odour-baited traps have been developed. These traps disperse the odour bait, and capture attracted mosquitoes using a fan-powered circulating airflow. A recent study showed that mass trapping using odour-baited traps in Kenya can greatly reduce malaria prevalence, and thus the system is now considered a viable vector control tool against malaria mosquitoes. To date, numerous studies have been performed on odour attraction, sensory cue combinations, and trap

these traps. We studied the flight dynamics of female malaria mosquitoes *Anopheles coluzzii* around a hanging and a standing odour-baited trap, with the aim to better understand mosquito flight behaviour around traps, as well as traps capture efficiency and dynamics. Our analysis of more than 2500 three-dimensional flight tracks shows that mosquitoes exhibit a highly stereotypic flight behaviour around the trap, whereby they are attracted to a specific region close to the capture funnel, but when detecting the inward airflow they quickly respond and therefore often avoid being captured. In addition, we found fundamental differences between the mosquito flight dynamics around the hanging and standing trap, implying a higher attractiveness of the standing trap and a more efficient capture mechanism of the hanging trap. This knowledge might aid in the improvement of current odour-baited traps, and could even lead to the development of novel trap designs.

A11.34 QUANTITATIVE ANALYSIS OF ENERGY BALANCE IN THE DYNAMIC SOARING OF STREAKED SHEARWATER

WEDNESDAY 5 JULY, 2017 13:50

YOSHINOBU INADA (TOKAI UNIVERSITY, JAPAN), NAOYA TAKAMURA (TOKAI UNIVERSITY, JAPAN), TAKAHIRO YOSHIKAWA (TOKAI UNIVERSITY, JAPAN), YOSHINARI YONEHARA (THE UNIVERSITY OF TOKYO, JAPAN), KATSUFUMI SATO (THE UNIVERSITY OF TOKYO, JAPAN)

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Streaked Shearwater *Calonectris leucomelas* is a species of seabird living in the north-western area of Pacific Ocean. The size of the adult bird is about 50 cm in total length and 120 cm in wingspan. The marked characteristic of its flight is the dynamic soaring, which is an economical flying method enabling long duration and long distance flight by utilizing wind energy. This study investigated the energy balance in the dynamic soaring of streaked shearwater by conducting wind tunnel experiments with approximate flight condition. The experimental model was made referring to the wing and body shape of the streaked shearwater. The body attitude and the wind speed were given appropriately mimicking a one cycle of the dynamic soaring obtained from a 9-axis sensor device attached to wild streaked shearwaters. In consequence, the acquisition of energy from the wind was quantitatively confirmed and this could cover about 15% of the energy consumption by drag. Although the full scale energy-free flight was not completed because the energy consumption by drag exceeded the energy acquisition by dynamic soaring, the dynamic soaring clearly contributed to the energy saving flight of streaked shearwater.

A11.35 WIND SHEAR ESTIMATION BASED ON DYNAMIC SOARING OF SEABIRDS

WEDNESDAY 5 JULY, 2017 14:05

YOSHINARI YONEHARA (ATMOSPHERE AND OCEAN RESEARCH INSTITUTE THE UNIVERSITY OF TOKYO, JAPAN), YUSUKE GOTO (ATMOSPHERE AND OCEAN RESEARCH INSTITUTE THE UNIVERSITY OF TOKYO, JAPAN), MASARU NARUOKA (JAPAN AEROSPACE EXPLORATION AGENCY, JAPAN), KATSUFUMI SATO (ATMOSPHERE AND OCEAN RESEARCH INSTITUTE THE UNIVERSITY OF TOKYO, JAPAN)

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Procelariiformes seabirds (albatrosses and shearwaters) can fly long distances for a prolonged time by dynamic soaring flight which enables them to utilize wind energy and sustainably fly with minimum flapping. Although these seabirds are thought to utilize the wind shear near ocean surface where wind speed increase with height, the wind shear experienced by the birds are rarely observed. In this study, we evaluated the wind shear profile from three-dimensional movement data of the dynamic soaring flight of streaked shearwaters (*Calonectris leucomelas*) breeding in North-east Japan. A device with nine-axis sensor, GPS (speed and direction), and pressure sensor (altitude) was attached to two streaked shearwaters in September 2016. Flight direction, altitude, and ground speed of the bird fluctuated periodically which coincided with the characteristic of dynamic soaring flight. Assuming that this ground speed fluctuation was due to the heading of the bird relative to the wind and also the change in wind speed with height, we estimated the wind shear profile that most likely explains the relation between flight direction, altitude, and ground speed of the bird for every five minutes. Typical wind shear profile in which wind speed increase with height was estimated in most cases and the shear was steeper in tail wind flight than in head wind flight. In some cases, discontinuity in wind shear was observed presumably due to separated flow behind waves and swells. We show the possibility of evaluating the wind shear profile hidden in the movement of dynamic soaring flight.

A11.36 GAZE DIRECTION DURING PURSUIT IN PEREGRINE FALCONS

WEDNESDAY 5 JULY, 2017 14:20

JAMES A WALKER (UNIVERSITY OF OXFORD, UNITED KINGDOM), GRAHAM K TAYLOR (UNIVERSITY OF OXFORD, UNITED KINGDOM)

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Peregrine falcons (*Falco peregrinus*) rely on aerial attack behaviours for predation using visual information to guide their trajectory to target interception. In order to understand how peregrines implement the underlying feedback law governing pursuit it is important to determine their gaze strategy in relation to both the visual environment and the target. Two possible strategies exist: a) directly tracking the target with continuous head movements (like the seeker of a guided missile) or b) fixing the head in space relative to the visual environment (like an image-stabilised camera) and tracking the target's image over the retina. To address this, we released peregrines trained to pursue and intercept a falconer's lure towed by a small remotely controlled aircraft. The birds were fitted with a head-mounted IMU to track head movements and a GPS device to track position during flight. We found that in the terminal phase of pursuit, peregrines continuously track the target position with their head rather than stabilising their gaze against the background. This suggests that peregrines use information gathered from the tracking movements of the head to feed back into the guidance law to intercept the target and has implications for the design of visually guided unmanned aerial vehicles.

A11.37 WHAT GOES UP MUST COME DOWN - BIOMECHANICS OF LANDING INSECTS

WEDNESDAY 5 JULY, 2017 14:35

SIMON V REICHEL (UNIVERSITY OF APPLIED SCIENCES BREMEN, GERMANY), SUSANNA LABISCH (UNIVERSITY OF APPLIED SCIENCES BREMEN, GERMANY), JAN-HENNING DIRKS (UNIVERSITY OF APPLIED SCIENCES BREMEN, GERMANY)

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The jumping movement of insect provides a fast and efficient means of locomotion and has drawn the interest of biomechanics research in the last years. However, the biomechanics of the actual landing are not yet clear. At take-off the insect cannot predict the parameters of the landing site. Unforeseen obstacles or different substrates require a flexible and highly dynamic landing performance. How do jumping insects control their landing? Are there active or passive mechanisms involved? To answer these questions we analysed the impact of free falling desert locusts using high-speed imaging and motion tracking. Experiments with different falling and landing parameters, such as angle and body temperature indicated that locusts are capable to actively and passively control their landing speed, posture and the first point of contact. Interestingly, alive locusts seem to actively increase their falling speed before impact. They also tend to land on the head while in cooled or dead locusts the proportion changes with dropping angle. This could indicate the presence of a biomechanical stabilisation mechanism of the head and pronotum. When dropped at different initial angles, alive locusts always turned their body head first before impact. Significant differences between living and dead locusts suggest that this

turning movement is also an active mechanism. Our results also show a characteristic bending movement of the locust abdomen. Occurring rarely in dead locusts this seems to be a passive control mechanism, possibly decreasing the time the locust needs to turn the body to an upright direction after impact.

A11.38 THE MECHANICAL FUNCTION OF THE BICEPS BRACHII AND SCAPULOTRICEPS MUSCLES OF THE PIGEON (*COLUMBA LIVIA*) DURING FLIGHT

📅 **WEDNESDAY 5 JULY, 2017** ⌚ **14:50**

👤 **LAURA A MCFARLANE** (UNIVERSITY OF LEEDS, UNITED KINGDOM), **GRAHAM N ASKEW** (UNIVERSITY OF LEEDS, UNITED KINGDOM)

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Modulation of bird flight performance requires complex changes in the wing kinematics but how the wing muscles function to produce these changes is unknown. In this study we determined the mechanical function of the pigeon biceps brachii and scapulotriceps muscles during take-off, slow flight and landing by simulating in vivo muscle length change and activity patterns in vitro using the work loop technique. The biceps brachii muscle is activated in late upstroke, absorbing energy that resists and decelerates elbow extension. The biceps brachii muscle actively shortens in the final half of the downstroke, generating the mechanical work to flex the elbow and depress the humerus. Over the course of the wingstroke the biceps brachii muscle generated zero net work. The scapulotriceps muscle is activated towards the end of the downstroke. This muscle is actively lengthened, absorbing energy and stabilising the elbow flexion at the end of the downstroke. During the upstroke the scapulotriceps muscle actively shortens, generating work that facilitates the extension of the elbow joint. The scapulotriceps generates relatively little net work, compared with the main power generating muscle, the pectoralis muscle. The differences in the net work generated by these muscles are due to differences in the timing of activation and strain that result in variation in the positive and negative work performed. Muscle mechanical function was consistent across the flight modes investigated in both the biceps brachii and scapulotriceps muscles.

A11.39 THE BIOMECHANICS OF DIPTERAN FLIGHT MUSCLES

📅 **WEDNESDAY 5 JULY, 2017** ⌚ **15:05**

👤 **JONATHAN W PAGE** (UNIVERSITY OF OXFORD, UNITED KINGDOM), **SIMON M WALKER** (UNIVERSITY OF OXFORD, UNITED KINGDOM)

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The remarkable flight ability of dipteran insects is afforded by a complex system of muscles responsible for controlling wing movements, though none are attached to the wings themselves. These muscles alter wing movements by changing the shape of the hinge that joins the wing to the thorax. Activity patterns of the control muscles, and the impact of these activities on wing movement, have been studied before, but examining the biomechanics through which these muscles induce these changes in wing kinematics is difficult using traditional techniques. We have therefore utilised synchrotron x-ray time-resolved microtomography to visualise the movement of these muscles during flight in *Calliphora vicina*. Our previous work compared the binary case of high- and low-amplitude wingbeats, and revealed muscle-specific differences in muscle strain amplitude, phase, and period. Furthermore, some muscles exhibit buckling at certain stages during the wingbeat, afforded by a large tendon that connects the muscle to the associated sclerite. When buckled, these muscles cannot exert force, suggesting that buckling can subvert the need to activate the muscle on each wingbeat at the appropriate time, which is metabolically expensive. Here, we greatly extend this work by making use of new datasets with greatly improved spatiotemporal resolution. This allows us to examine how steering muscle strains change across the continuous spectrum of wing movements available to flies. Our study has therefore continued to elucidate some of the biomechanics of the dipteran flight motor, and illustrate the engineering principles associated with the incredible flight ability of these insects.

A11.40 FUNCTIONAL IMPLICATIONS OF ARCHITECTURAL GEAR RATIO WITHIN A COMPARTMENTALIZED MUSCLE

WEDNESDAY 5 JULY, 2017 15:45

CHRIS TIJS (CONCORD FIELD STATION - HARVARD UNIVERSITY, UNITED STATES), NICOLAI KONOW (DEPARTMENT OF BIOLOGICAL SCIENCE - UNIVERSITY OF MASSACHUSETTS LOWELL, UNITED STATES), ANDREW BIEWENER (CONCORD FIELD STATION - HARVARD UNIVERSITY, UNITED STATES)

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Pennate muscles can decouple shortening speed of the fibers (V_{fiber}) from that of the whole muscle (V_{muscle}) through fiber rotation, expanding a muscle's performance repertoire. Architectural gear ratio ($\text{AGR} = V_{\text{muscle}} / V_{\text{fiber}}$) has been determined for a single muscle region, which assumes homogeneous fiber mechanics, and has mainly been investigated for supramaximal excitations, although muscles are recruited submaximally *in vivo*. The pennate rat medial gastrocnemius (MG) is compartmentalized with proximal fibers that are shorter and more angled than distal fibers, raising the question of whether uniform AGR exists within this muscle. To quantify AGR, we used sonomicrometry to measure *in situ* supramaximal ($n=8$) and submaximal ($n=6$) force-velocity properties of three MG structural units in anesthetized rats: the whole muscle, proximal fibers and distal fibers. Isotonic shortening contractions controlled and measured by a servomotor were obtained at various force levels. Speed was determined for each structural unit at the time point averaged isometric optimum length of proximal and distal fibers was reached. AGR was calculated for each force level, and for proximal (AGR_{prox}) and distal (AGR_{dist}) fibers separately. During supramaximal excitation, AGR_{prox} (1.82 ± 0.40) was higher ($p = .027$) than AGR_{dist} (1.35 ± 0.25) with no differences for submaximal stimulation (1.74 ± 0.40 and 1.40 ± 0.30 , respectively; $p = .157$). Increase in MG force level caused AGR to decrease ($p < .05$), although to a limited extent ($< 20\%$). These findings suggest that various muscle regions can show distinct fiber mechanics, but that these differences are reduced at submaximal activation levels. This may be important in considering regional muscle heterogeneity *in vivo*. Funded by NIHAR055648 to A.B.

A11.41 INTEGRATION OF JAW AND TONGUE MOVEMENTS, AND TONGUE CONTROL OF FOOD DURING AXOLOTL CHEWING

WEDNESDAY 5 JULY, 2017 16:00

NICOLAI KONOW (UMASS. LOWELL, UNITED STATES), EGON HEISS (U. JENA, GERMANY), FLORIAN WITZMANN (MUSEUM FÜR NATURKUNDE BERLIN, GERMANY), ELIZABETH L BRAINERD (BROWN UNIVERSITY, UNITED STATES)

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Aquatic feeding vertebrates must balance risks of food escape with requirements for food processing before swallowing. Food control in water is challenged by the lack of appendages and fleshy tongues, and instead depends on the hyoid apparatus acting as a hydrodynamic piston to produce fine-scale intraoral water flows that

control food positioning before the toothy intraoral surfaces engage in the chewing power stroke. The deep and hidden placement of the musculoskeletal elements involved can complicate measurements of tongue, jaw and food movements during chewing. Therefore, we used biplanar fluoroscopy to measure skeletal movements (XROMM), muscle length-change behaviors (fluoromicrometry) and food positioning in Axolotls ($N=6$) chewing on crickets. The data were used to test an idea arising from earlier EMG studies; that muscular-actuated tongue motion moves food caudally during gape opening, likely to avoid its escape. During the chewing preparatory phase, we measured sternohyoid shortening resulting in caudoventral hyoid excursion before most chews, but some chews involved sternohyoid lengthening and rostral hyoid excursion before or during gape opening. Cross-correlation analyses revealed that intraoral food movement lagged from the initiation of tongue movements, consistent with the idea that the tongue moves water to exert food displacement. Our data underscore problems associated with predicting hidden motion based on EMG and suggest an unappreciated diversity in salamander chewing movements. Ongoing work uses thyroxin-induced metamorphosis of the same subjects into terrestrial organisms to determine the changes in tongue and jaw morphology and movements that are associated with transitioning to feeding on land.

A11.42 HOW DOES PHARYNGEAL STREAMLINING AFFECT SUCTION FEEDING DYNAMICS IN FISHES?

WEDNESDAY 5 JULY, 2017 16:15

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To capture prey by suction, fish generate a flow of water that enters the mouth, and exits at the back of the head. It was previously hypothesized that a streamlined shape of the posterior pharynx and the pectoral region of the body are important to enable an unobstructed outflow with minimal hydrodynamic resistance. However, due to the lack of optical access into the pharyngeal cavity, and the limitation of biomechanical models, this hypothesis remained untested. Using a recently published computational model that allows a dynamic simulation of both inflow and outflow of water, we quantified the effects of different shapes of the posterior pharynx on the dynamics of suction feeding. The kinematic input in the model was based on a representative feeding act of a percomorph species (*Lepomis gibbosus*). It showed that modifying a wedge-shaped protrusion of the pharynx near the region of the oesophagus entrance, previously hypothesized to be optimal, into a straight surface perpendicular to the incoming flow has a negligible effect on the dynamics of suction feeding. With the help of graphical reconstructions based on CT-scans, we further evaluate whether or not aspects of the actual three-dimensional shape of the outlines of the buccopharynx point to a role in streamlining suction flows.

A11.43 INTRAORAL FOOD PROCESSING IN THE NEWT *TRITURUS CARNIFEX*: HOW DO THEY CHEW?

WEDNESDAY 5 JULY, 2017 16:30

EGON HEISS (FRIEDRICH-SCHILLER-UNIVERSITY OF JENA, GERMANY), DANIEL SCHWARZ (FRIEDRICH-SCHILLER-UNIVERSITY OF JENA, GERMANY), NICOLAI KONOW (UNIVERSITY OF MASSACHUSETTS LOWELL, UNITED STATES)

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Vertebrate feeding systems have evolved remarkably diverse specializations that allow exploitation of a great variety of food sources. Besides the initial food acquisition, intraoral processing, i.e. mechanically reducing food within the mouth, or chewing, has played a major role in evolutionary adaptive procedures to successfully exploit food sources in a given trophic environment. Processing mechanisms are known for all major vertebrate clades, from fishes to mammals, but form and function of the processing apparatus to crush, grind, or puncture food items can differ substantially between and within major groups. However, rhythmic and coordinated cyclic movements of skull, jaws and hyobranchial elements appear to be a common trait. While processing mechanisms in amniotes (sauropsids and mammals) and fish-like vertebrates have been subject of intense research, processing mechanisms in lissamphibians remain relatively unstudied, resulting in the common perception that lissamphibians simply do not chew and instead swallow prey whole. Here, we present the first results from behavioral observations, high-speed x-ray videos and anatomical analyses of an undescribed intraoral processing mechanism employed by *Triturus carnifex*. The salamandrid newt *T. carnifex* displays a conspicuous behavior following prey-capture, involving rhythmic head bobbing, coordinated with cyclic gape and hyolingual movements. Our x-ray recordings reveal that, rather than chewing prey between upper and lower jaw elements as typically seen in other tetrapods, *T. carnifex* processes prey by rasping it against its palatal dentition. We compare the processing mechanism of *T. carnifex* with processing in fishes and amniotes and discuss it in an evolutionary context.

A11.19 CAN MODIFYING THE SOLAR SURFACE OF HORSES' HOOVES IMPROVE DISTAL LIMB IMPACT VIBRATION DAMPING?

WEDNESDAY 5 JULY, 2017 POSTER SESSION

AMY L BARSTOW (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), RENATE WELLER (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), THILO PFAU (ROYAL VETERINARY COLLEGE, UNITED KINGDOM)

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The foot-surface impact is a key event in the stride cycle, which provides opportunities to apply external interventions to alter shock absorption and load distribution in horses. Such interventions have the potential to optimise competition performance and safeguard welfare by reducing injury risk. Foot-surface impacts result in rapid deceleration of the horse's foot and impact vibrations are subsequently transmitted through the equine distal limb. On firm

surfaces these parameters are of greater magnitude than on a softer, deformable surface which can act to damp the effects of impact. However, a large number of horses must conduct, some or all of, their exercise upon firm surfaces (e.g. roads). We propose that the use of modern 'sole-packing' materials, applied to the solar surface of horses' hooves, may provide an alternative method of damping the effects of impact. To investigate this 12 horses were equipped with a hoof mounted, high range accelerometer. Horses, shod in plain steel shoes, both with and without a sole-packing material, were trotted in-hand over a firm concrete surface. Six foot-surface impacts per horse per condition were selected for analysis. A fast Fourier transform was applied to the accelerometer output from impact for 30 milliseconds, and vibration frequency and power parameters were extracted. Preliminary results suggest that sole-packing materials have a greater effect upon reducing vibration power compared with vibration frequency. Sole-packing materials may, therefore, have the ability to damp impact vibrations and further analysis is required to determine the scope of their damping properties.

A11.20 A TENDON-CY TOWARDS SPEED: HOW LOADING AFFECTS THE VELOCITY OF TENDON RECOIL

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The fastest movements in the animal kingdom achieve mechanical power outputs that far exceed the mechanical power capacity of skeletal muscle. In order to amplify mechanical power, muscles slowly contract to stretch tendons and store potential (elastic) energy. When tendons recoil, the stored energy is rapidly released to amplify mechanical power. To function in amplifying power, tendons must recoil at high-speeds. However, most studies characterize the mechanical properties of tendons at slow rates following prescribed length trajectories. We predict that the velocity and power output of a recoiling tendon depends on the load that is being accelerated. To test this prediction we isolated the tendon tissue from bullfrog plantaris muscle-tendon unit. We then applied a 2.5% stretch to the tendon before rapidly unloading the tendon to measure the speed of recoil and rate of energy release. We measured the maximal recoil speed of unloaded tendon to be 3.36 ± 0.0795 (L/L_r). As we increased tendon load, we observed a decrease in the maximal recoil velocity. These data are consistent with mathematical models that suggest tendon contributions to power may be limited by the size of an animal.

A11.21 RECONSTRUCTION AND MUSCULOSKELETAL MODELLING OF THE PELVIC AND HINDLIMB MUSCULATURE OF THE FOSSIL SALIENTIAN *TRIADOBATRACHUS*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

LAURA B PORRO (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), ENRICO A EBERHARD (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), AMBER J COLLINGS (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), CHRISTOPHER T RICHARDS (ROYAL VETERINARY COLLEGE, UNITED KINGDOM)

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The uniquely derived body plan of frogs is considered to be adapted for jumping; nonetheless, modern frogs display diverse locomotor behaviours. The earliest fossil stemanuran, the Early Triassic *Triadobatrachus massinoti*, exhibits a mosaic of features representing a key transitional stage in the evolution of the anuran *Bauplan*. The locomotor capabilities of *Triadobatrachus* - and whether it was capable of jumping - has been the subject of numerous previous studies yet remains unresolved. We identified osteological correlates of homologous muscle attachment sites in the extant phylogenetic bracket of *Triadobatrachus* - frogs and salamanders - from contrast-enhanced CT scans of over 20 taxa spanning wide phylogenetic and locomotor ranges, as well as information from the literature. These data, along with CT scans of *Triadobatrachus*, were used to rigorously reconstruct its pelvic and hindlimb musculature in 3D. We found that the musculature of the pelvis and thigh strongly resembles that exhibited by extant frogs, while the more distal hindlimb musculature is an amalgam of the anuran and caudate conditions. These findings suggest that selective pressures driving the morphofunctional evolution of frogs may have acted earlier and/or more strongly on the proximal hindlimb than distal elements. We will apply our musculoskeletal reconstruction of *Triadobatrachus* to our existing frog template model to rigorously test the locomotor performance of *Triadobatrachus* and shed light on the evolution of locomotion at the base of Anura.

A11.22 JUMP OPTIMISATION OF A MORPHABLE MUSCULOSKELETAL MODEL: PROBING PERFORMANCE AGAINST CHANGING HIND-LIMB PROPORTIONS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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In the evolution of anurans, a general lengthening of the hindlimbs may have helped increase jumping performance. The proportionality of leg segments also changed; compared with Paleozoic amphibians, in many cases the tibiofibula lengthened more than the femur. How did this transition of proportions impact range of motion and extension? What was the effect on jump strategy and performance?

To address such implications, a musculoskeletal model of *Kassina maculata* was developed from micro-CT scanning and dissection, implemented in physics framework MuJoCo. The model includes pelvic and hind-limb musculature and is parametrized to be 'morphable'; a set of theoretical anatomies was created with the tibia ranging between 80%-120% original length, with the femur adjusted to preserve total leg length. From live jumping trials, recorded skin marker coordinates were converted into joint kinematics to drive the model. Total extension distance was maximal in the original anatomy, decreasing by 6% for shortest tibias and <1% for longest tibias. Inverse dynamics with a constrained foot contact showed required knee torques and knee extensor moment arms scale proportionally with tibia length, while hip torques were mostly unaffected. This suggests that long tibias increased work potential without strongly affecting extensor load. Joint kinematics would unlikely remain the same after morphological changes, as animals would adapt their behaviour. An optimisation algorithm using iterative forward dynamic simulations is being developed to find the best stance and muscle control for a given model. This may uncover jump strategies in transitional anatomies that gave early anurans their evolutionary edge.

A11.23 ARE CRAWLING HUMANS MORE LIKE HORSES OR HIPPOS?

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The symmetrical gaits of quadrupeds can be described using a 2-figure formula consisting of the hindlimb duty factor and relative timing (phase) of the ipsilateral forelimb. Despite the high number of possible duty factor-phase combinations, relatively few gaits are seen in nature, raising the question as to why terrestrial quadrupeds use the gaits that they do. Recent work suggests that distinct groups of animals use specific duty factor-phase combinations at walk, which are concomitant with low mechanical work. When considering human crawling, neuro-mechanical and developmental explanations are commonly given for the quadrupedal gaits of human adults and children; however, here we suggest that the mechanical model for duty factor-phase relationships provides an alternative explanation for the crawling gaits of humans. Human subjects were asked to crawl at their preferred gait at a range of speeds before being asked to crawl with different limb sequences/phases. Results show that adult humans largely comply with the mechanical model for quadrupedal walking gait selection, though fall into two of the groups of animals seen in the literature: the group containing horses (and other medium-large mammals) and the group containing hippos (and other slow, slow-muscled and small animals). The subjects were asked to rate the difficulty of achieving the alternative phases and the majority found the phases not seen in nature (and modelled to demand the most mechanical work) were most difficult to achieve. Here we propose an alternative theory for crawling gait selection in humans, consistent with low mechanical work rather than neurological control.

A11.24 CORAL VS. COMPUTER: VALIDATING A PULSING POLYP SIMULATION USING FLOW VELOCITY FIELDS, VORTICITY, AND LAGRANGIAN COHERENT STRUCTURES

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Xeniid corals increase the local flow around their colonies through the collective pulsing behaviour of their polyps. This pulsing behaviour is thought to increase mass transfer of nutrients and gas exchange between the organism and its environment. At previous meetings, we presented the first descriptions of the flow fields around pulsing polyps and qualitatively compared actual flow data to both 2D and 3D immersed boundary simulations of polyps. Here, we investigate the flow fields in a quantitative manner using particle image velocimetry (PIV) data, 3D immersed boundary simulations, and Lagrangian coherent structure (LCS) analysis. We first quantitatively compare velocity fields and vorticity of PIV data from a single polyp to its simulated counterpart. For this comparison, we do not limit ourselves to the sagittal plane of the polyp but study several slices, both horizontal and vertical, spanning the width and height of the polyp head; this allows us to generate a more complete 3D understanding of the flow space around a polyp. Secondly, we look for LCSs in both the collected and simulated data. LCS analysis can inform us on boundaries between mixing regions and regions of inflow/outflow; particles found in one LCS might be restricted to a certain region of the flow field and never trade places with particles from another structure. Being able to match LCSs in our simulations with observed LCSs means our immersed boundary model could be used to simulate particle exchange in pulsing corals.

A11.44 HOW HEAD AND BODY MUSCLES MEET THE POWER DEMANDS OF SUCTION FEEDING IN BLUEGILL SUNFISH

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Powerful vertebrate feeding behaviors pose a challenge: a muscle's work (energy) and power (rate of energy) are limited by its mass, yet cranial muscles are a small proportion of total muscle mass. To meet this challenge, animals can increase feeding musculature mass to increase work and power, and/or amplify muscle power by storing then rapidly releasing muscle energy. To produce powerful suction

feeding, at least one fish (largemouth bass) uses its massive body muscles to power the rapid mouth expansion needed to accelerate water and food into the mouth. While body muscle power is likely important for species that share the large mouth and fusiform body of bass, fish with different morphologies may rely on relatively larger cranial muscles or power amplification. We examined bluegill sunfish, a closely-related species with a small mouth, tall and laterally-compressed body, and a relatively large cranial muscle, the sternohyoideus. We measured intraoral pressure and volume changes to calculate the power and work of suction expansion, and measured muscle shortening to confirm both axial muscles and the sternohyoideus generated power during suction feeding. Cranial muscles alone cannot produce the most powerful strikes, which would require power and work outputs likely exceeding their capacity: >2,000 W/kg and >80 J/kg, respectively. In contrast, the body muscles could generate all strikes without exceeding 450 W/kg power or 6 J/kg work. Thus, fish with different body and mouth shapes can use the large body muscles as part of the feeding apparatus to meet the power demands of suction feeding.

A11.45 LAB VERSUS FIELD: FEEDING KINEMATICS OF POLYPHENIC BLUEGILLS (*LEPOMIS MACROCHIRUS*)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Polyphenic populations are a valuable resource for understanding the relationship between form and function. Bluegill sunfish from Lake Waban, Massachusetts exhibit variation in their trophic morphology and diet based on habitat. Littoral bluegills have wider pharyngeal jaws and feed on a variety of benthic invertebrates. Pelagic bluegills are constrained by their smaller mouth size to feed almost exclusively on cladocerans. We investigated the kinematics and suction pressure of these ecomorphs when feeding on three prey types. We hypothesised that littoral bluegills would use more suction and capture their prey faster because they typically feed on grasping invertebrates. Instead, there were no differences in peak subambient pressure or feeding kinematics between ecomorphs within a prey type. Within an ecomorph, bluegills used less pressure and had a smaller mouth gape when capturing brine shrimp. Pelagic bluegills did not vary their ram distance across all prey types. This suggests that pelagic fish are less flexible in their prey approach behaviour due to their zooplankton specific diet. Furthermore, we also recorded feeding behaviours of bluegills in the field and compared their kinematics to those recorded in the lab. Wild bluegills in the littoral zone used more ram than captive bluegills. Both ecomorphs approached their prey slower in the field. Therefore, these results suggest that bluegills are modifying their behaviour as a result of feeding in the lab. Future studies will compare the enzymatic activity of the feeding muscles to determine if there is variation in aerobic or anaerobic capacity between littoral and pelagic bluegills.

A11.46 BIOMECHANICAL PARAMETERS OF THE JAW CARTILAGE OF THE BIG SKATE (*RAJA BINOCULATA*) – A MATTER OF SHAPE?

WEDNESDAY 5 JULY, 2017 POSTER SESSION

• PETRA DITSCH (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES), EVELINA NATEKIN (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES), TONY LIANG (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES), CHERYL WILGA (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES)

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Raja binoculata is the largest skate species of North America. It inhabits the ocean bottom from intertidal to continental shelf and feeds on shellfish, worms and crabs. As typical for all Batoidea, the jaws are composed of tessellated cartilage, the upper jaws are not directly connected to the cranium giving them more freedom of movement, and the hyoid is broken up (euhyostylic). In this study we use 3D scanning, 3D printing and mechanical testing to understand how *R. binoculata* crushes hard shelled prey. Due to the shape of the jaws, the second moment of area is considerably higher than for cylindrical shapes with the same cross sectional area. Our results also show that the flexural stiffness of the jaw elements is significantly increased relative to the cylindrical shape. Both parameters indicate that shape is optimized to withstand the forces experienced during crushing. In addition to quantifying compressive strength, strain and E-Modulus of the jaw elements of *R. binoculata*, we investigate the effect that shape has on the mechanical variables measured. In contrast to engineered shapes, biological shapes are not standardized along geometric parameters. Nevertheless, biologists use the same methods to measure biomechanical parameters to assess the performance of biological shapes. Using 3D-prints of similar synthetic material as cartilage, with the same shape as the jaw elements, we found that shape can impact the biomechanical properties measured.

A11.47 AVIAN WING CONFIGURATIONS IN AND OUT OF GROUND EFFECT

WEDNESDAY 5 JULY, 2017 POSTER SESSION

• JORN A CHENEY (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), NICK E DURSTON (UNIVERSITY OF BRISTOL, UNITED KINGDOM), JONATHAN P J STEVENSON (UNIVERSITY OF BRISTOL, UNITED KINGDOM), JIM R USHERWOOD (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), SHANE P WINDSOR (UNIVERSITY OF BRISTOL, UNITED KINGDOM), RICHARD J BOMPHELY (ROYAL VETERINARY COLLEGE, UNITED KINGDOM)

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Birds are the only taxon to have evolved powered flight from a non-membrane wing. The reduced avian wing skeleton would seemingly limit the complexity of wing morphing, but numerous degrees of freedom arise from the dense array of protruding feathers, allowing for both continuous and discontinuous surface configurations. Wing morphing capacity is what allows birds to achieve diverse manoeuvres in unsteady and complex environments. By studying wing configurations adopted during fundamental and repeatable flight behaviours, we can gain insight into morphological limitations

of the avian control surfaces and flight stability. In this work, we chose to study relatively-steady wing configurations during two gliding behaviours: in and out of ground effect. We observed multiple trials of five birds: a raven (*Corvus corax*), barn owl (*Tyto alba*), tawny owl (*Strix aluco*), goshawk (*Accipiter gentilis*), and tawny eagle (*Aquila rapax*) as they flew down a 20m corridor that constrained their flight to a straight path. We used videogrammetry with high-speed cameras placed above and below the birds to reconstruct their wing surfaces. We combined these detailed surfaces with tracked dynamics of anatomical landmarks using a motion capture system. Gliding in ground effect enhances lift and reduces drag, so differences in wing configuration must account for this if the centre of mass trajectory is to remain consistent despite varying proximity to the ground. Using our surface reconstructions, we report on wingspan, wing twist, tail orientation, glide speed, glide angle and the steadiness of birds gliding in still air.

A11.48 A WAY TO AERIAL BEHAVIOURS: WIRELESS NEURAL TELEMETRY IN FREELY FLYING DRAGONFLIES

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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In evolutionary history, insects were the first animals to fly. Not surprisingly, many interesting and essential behaviours such as foraging, courtship, and migration happen in the air. Generations of experimental biologists have been studying these behaviours and derived rules that govern them. However, to understand the neuronal implementations of these rules, we must monitor the activity in relevant circuits during behaviour on an event-to-event basis. Currently, behavioural studies on flight control are often limited by tethering the insect which constrains its flight dynamics. Recent developments of neural telemetry takes our experiments to the air. Using a 50mg insect backpack, we have successfully recorded a variety of neural signals from a flying dragonfly in two parallel projects. Firstly, we measured the activity of visual interneurons specifically tuned to small targets. These neurons are thought to support flight steering during prey interception, although direct evidence as to how they are involved is still missing. Our wireless recordings of hunting dragonflies show the neurons' responses to incoming prey before takeoff and to certain in-flight events, suggesting a role in flight planning and error detection. Secondly, the structural properties of the dragonfly wings have been frequently linked to the flight mechanics. The wings are adorned with mechanosensors that could provide necessary feedback for flight control. We will present preliminary recordings of a group of campaniform sensilla from a flying dragonfly. These ongoing projects directly link dragonfly flight to sensory encoding and will provide insights into the evolution of insect wing design and flight mechanics.

A11.49 THE FELLOWSHIP OF THE WING: HOMING PIGEONS (*COLUMBA LIVIA*) SIGNIFICANTLY INCREASE THEIR WINGBEAT FREQUENCY WHEN FLYING IN PAIRS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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One of the most commonly cited reasons for travelling as a group is to reduce energy expenditure and enhance locomotor performance. Indeed, birds flying in V-formations save significant amounts of energy. By contrast, pigeons (*Columba livia*) flying in close cluster flocks have been shown to increase their wingbeat frequency by 0.1 Hz when flying near to or behind other birds. However, these differences have not been measured relative to flying solo on an individual level. Our study addresses this question by quantifying changes in flight characteristics of homing pigeons flying solo and in a pair. Birds were tracked with 5 Hz GPS and 200 Hz tri-axial accelerometers. We found that, even after accounting for the effects of wind support and crosswind, birds flying in pairs increased median wingbeat frequency by 1.0 Hz (18.1%) relative to flying solo. Furthermore, differences in tarsus length (body size) between birds had no effect on wingbeat characteristics. Our results show that, for pigeons, the magnitude of the energetic cost of flying together is substantially higher than previously thought. The 1.0 Hz increase in wingbeat frequency - ten times the increase seen in previous work - suggests that there is an additional, significant cost to flying in a flock, the majority of which comes merely from the act of flying with another individual, rather than the relative position of the bird within the flock or the size of their partner.

A11.50 HOW DOES BODY STIFFNESS MODULATION AFFECT UNDULATORY SWIMMING? SOFT SENSORS CAPTURE FIN CURVATURE FOR A CLOSED LOOP SOFT ROBOTIC FISH

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The most important mode of locomotion observed in fishes is Body- Caudal-Fin undulation. Although numerous studies of body kinematics and muscle activity patterns have generated essential knowledge on the mechanics of swimming, some key parameters, such as how the extent of bilateral muscle activation affect propulsive performance, remain under-explored due to the difficulty to experimentally manipulate muscle activation in

freely-swimming fishes. To gain insight in axial co-contraction, we manufactured actively-controlled pneumatic actuators in a soft robotic fish. To close the loop we measured fin curvature with sensors made of hyperelastic silicone elastomers with embedded micro-channels containing liquid metal. When the fin is curved as soft actuators are pressurized, strain is applied to soft sensors, thus increasing the overall length of the microchannel, and changing the electrical resistance. Fin curvature can then be correlated from measuring changes in resistance in response to bending. We measured thrust production at undulation frequencies ranging from 0.3 Hz to 1.2 Hz in a recirculating flow tank at flow speeds of up to 28 cm/s. This system generated more thrust at higher tail beat frequencies. Self-propelled speed was found to be 0.8 foil lengths per second at ~13 cm/s. The physical model is capable of producing substantial trailing edge amplitudes with maximum excursions equivalent to 1.4 foil lengths, and of generating considerable thrust. Altering the extent of bilateral co-contraction in a range from 17% to -22% of the cycle period showed that thrust was maximized with some simultaneous bilateral co-contraction of ~3% to 6% of cycle period ($p < 0.05$).

A11.51 VARIABILITY AMONG FOOTPRINTS OF CICONIA BOYCIANA (*AVES: CICONIIDAE*) IN HOMOGENEOUS SEDIMENT

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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We conducted an ichnological experiment to reveal morphological variability among *Ciconia boyciana* (Oriental stork) tracks left on wet potter's clay. We obtained 54 footprints from two individuals, and measured the area, length, width, depth, volume, and rotation for each track. The birds were also filmed while walking. One anatomical feature unique to *C. boyciana* is that unlike other wading birds, it does not leave metatarsophalangeal pad impressions. This feature can be used to distinguish *C. boyciana* tracks from those left by other birds with similar body weight and habitat. Track width varied by up to 30%, with wider tracks (digits splayed) found in trackways with shorter, wider steps. Conversely, narrow trackways with a large stride length were comprised of tracks with the toes closer together. Coefficient analysis of track geometry reveals that the width and depth of footprints vary inversely to maintain a consistent volume. Placing the *C. boyciana* footprints into a theoretical track morphospace (for avians with four digits) indicates that most footprints are deeper between digits III and IV than between II and III. Combined with video footage which revealed that *C. boyciana* moves its hips from side by side while walking, and that the footprints within any given trackway were outwardly rotated, we interpret this track morphology as resulting from a laterally directed pressure, exerted primarily through Digit IV.

A11.52 PRONATION AND SUPINATION: THE IMPORTANCE OF WING ROTATION TO INSECT FLIGHT

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

👤 MADELEINE R INGLIS (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), RICHARD J BOMPHELY (ROYAL VETERINARY COLLEGE, UNITED KINGDOM)

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The motion of an insect wing stroke cycle can be broken down into four key stages. These are two translational phases, the downstroke and upstroke, punctuated by two rotational phases, supination and pronation, during which the wings rapidly rotate and reverse direction in order to set an appropriate angle of attack for the translational phases. Peak forces are typically produced during the two translational phases, where the wing sweeps through the air with a high angle of attack. Evidence gathered from live insects has shown that flies adjust the timing of rotation during flight, and that this may account for enhanced lift forces that have been measured, but which cannot be attributed to conventional lift during the translation phases. This investigation explores the parameters that lead to enhanced lift during wing rotation, inspired by recently described phenomena from mosquitoes. Computational Fluid Dynamics simulations are used to model an idealised mosquito wing moving through air. Specifically, we perform a parameter sweep to characterise the aerodynamic effects of timing, speed and axes of wing rotations and how they can be optimised to produce unconventional aerodynamic forces. The wing structure is a simplified interpretation of a mosquito wing with similar thickness, chord length and wing length.

A11.53 HOW DOES MUSCLE ACTIVITY CHANGE WITH WATER DEPTH IN DOGS WALKING ON AN UNDERWATER TREADMILL?

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 ALISON P WILLS (HARTPURY UNIVERSITY CENTRE, UNITED KINGDOM), SIMON PARKINSON (HARTPURY UNIVERSITY CENTRE, UNITED KINGDOM), JANE WILLIAMS (HARTPURY UNIVERSITY CENTRE, UNITED KINGDOM)

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Canine rehabilitation is a rapidly developing area of veterinary medicine with an increasing range of techniques such as physical therapy, massage and hydrotherapy becoming widely available. The aim of these therapies is to restore animals to full health post-operatively, manage long-term conditions and promote fitness. Equine and canine kinematic research has found a relationship between water depth and kinematic parameters, however, there is limited research assessing the change in muscle activity with increasing water depth. The aim of this study was to investigate the impact of water depth on the muscle activity of dogs when walking on an underwater treadmill (UWT). Surface electromyography was used to assess muscle activity of the gluteus medius (GM) and longissimus dorsi (LD) of clinically sound dogs (n = 7) that were habituated to the UWT. Muscle activity was recorded at water depths of no submersion, above the tarsus, above the stifle

and the midpoint between the stifle and the greater trochanter. Kinematic measurements were performed to calculate motion cycle sequencing. There was a significant decrease ($p < 0.01$) in muscle activity between the depth above the tarsal and depths above the stifle, and at the midpoint between the tarsal and the greater trochanter in both the GM and LD. This suggests that UWT exercise at a water depth directly above the tarsal results in the greatest GM and LD muscle activity. These findings may help to inform future exercise and rehabilitation protocols for UWT exercise in dogs.

A11.54 PINE CONE SEED SCALES AS ROLE MODELS FOR ADAPTIVE FLAPS IN ARCHITECTURE

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

👤 SIMON POPPINGA (PLANT BIOMECHANICS GROUP FREIBURG, GERMANY), DAVID CORREA (SCHOOL OF ARCHITECTURE UNIVERSITY OF WATERLOO, CANADA), ACHIM MENGES (INSTITUTE FOR COMPUTATIONAL DESIGN UNIVERSITY OF STUTTGART, GERMANY), NIKOLAUS NESTLE (BASF SE ADVANCED MATERIALS AND SYSTEMS RESEARCH, GERMANY), BERND BRUCHMANN (BASF SE ADVANCED MATERIALS AND SYSTEMS RESEARCH, GERMANY), THOMAS SPECK (PLANT BIOMECHANICS GROUP FREIBURG, GERMANY)

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This project among biologists, material scientists/chemists and architects covers basic investigations of biological movement principles (especially hygroscopic pine cone motion) and their transfer into 3D-printed biomimetic structures for architecture. Pine cone seed scales are functionally very robust. We have shown that even fossilized scales from the Middle Miocene can still move. Such natural compliant structures serve as inspiration for technical, moving devices with low maintenance requirements. We performed comparative kinematical and anatomical analyses to gain insights into the functional morphology of pine cone seed scales from various pine-species. Manipulative experiments with the different tissues, which are involved in the hygroscopic behaviour, allowed for evaluation of their individual role in the motion. By letting cones and single scales dry out in a μ -CT scanner, we obtained time lapse recordings of the respective motions and thereby could gain information on the spatial deformation of the single tissues during the movement. By using novel 3D printing techniques with ABS plastic as resistance material and copolyester with embedded cellulose fibrils as hygroscopically active layer, we could print biomimetic flaps with tailored geometry and responsiveness. In addition to pine cone inspired bending movement also several other types of plant movement principles were successfully implemented into the printed structures - bilayer actuation, edge growth, kinematic amplification with curved folds, and snap-through instabilities. Our results prove that we have just opened nature's toolbox for the technical implementation of robust, fast or slow movement phenomena.

A11.55 SCALING OF CUTTING FORCES IN LEAF-CUTTER ANTS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Leaf-cutter ants are one of the top herbaceous consumers throughout the neotropics and play an important role in terrestrial ecosystems. The diverse tasks arising in the colonies include the maintenance of a fungus garden, which is fed with leaves cut in the surroundings of the colonies. Leaf-cutting is one of the most metabolically expensive activities insects engage in, and it hence becomes a crucial question, which of the polymorphs should be assigned to cutting in order to minimise metabolic costs at the colony level. In order to address this question, we measured the steady-state cutting forces of mandibles isolated from representatives of all castes from *Atta cephalotes* ants using a custom-built force transducer, and parafilm as a model substrate. While the cutting forces of mandibles of all castes were smaller than those of pristine razor blades, cutting forces increased with mass^{1/3} within workers, indicating that cutting forces may be proportional to a linear dimension of the mandibles such as the radius of the cutting edge. However, mandibles of soldiers which do not partake in leaf-cutting showed cutting forces comparable to those of mandibles of the smallest workers, suggesting that the increase of cutting forces among workers might be a result of mandible wear. While this result indicates that minimising mandible wear may be of key importance, the increase of the cutting force is still smaller than the increase in muscle force predicted from isometry, so that larger workers may still be better suited to perform cutting.

A11.56 POWER MINIMIZING STRATEGIES FOR CHANGING SPEED IN MICE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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When running, animals typically change speed by varying stride frequency or stride length, or a combination of both. However, the reduction in stance time and increase in push-off force required to achieve the higher stride frequencies and longer aerial phases necessary for higher speeds is undesirable for small animals such as mice, whose cost of locomotion is driven by muscle contraction power requirements. This poster will demonstrate how running mice instead primarily increase speed by becoming more crouched, increasing their stance length up to twice their leg length with little reduction in stance time. We show that the change in cost of increasing speed using this strategy is much less than if a mouse were to increase its stride frequency or push-off force. The disadvantage of a more crouched posture is that it reduces effective mechanical advantage (EMA), so that the muscles of crouched animals must exert more force to support their body weight than upright animals. However, by comparing morphological measurements from a range of species grouped by posture, we find that plantigrade animals such as mice may be able to achieve similar EMA when crouched as larger animals at a more upright posture. Additionally, at high stance angles, changes in posture have a negligible effect on EMA and thus the cost of body support. This may provide insight into why metabolic cost seems to vary relatively little with speed in smaller animals, and why smaller animals display much less distinction between gaits than larger animals.

A11.57 EFFECT OF SAMPLE PREPARATION ON THE MECHANICAL PROPERTIES OF ARTHROPOD CUTICLE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Cuticle is a natural, lightweight composite that forms the exoskeleton of arthropods: it is present in ~66% of all known species on Earth. Nanofibres of chitin (a long-chain polysaccharide) occur within a structural protein matrix (fibrous proteins that increase stiffness or elasticity). Cuticle has many diverse functions including: providing protection, giving structural support, controlling water content, mastication, penetrating prey cuticle with fangs, adhering to surfaces for climbing, forming wings for flight and sensory perception. As a result cuticle displays a remarkable range of properties, for example, elastic modulus varies over seven orders of magnitude, yet the basic macrostructure and composition of cuticle

is consistent across all arthropods. As such, it is a valuable material for biomimetic design. The chitin-protein fibres are arranged in helicoid layers, themselves incorporated into three ultrastructural layers: epicuticle, exocuticle and endocuticle, each of which possesses different properties. *In situ* imaging of crack propagation during mechanical testing would highlight how this structure affects fracture toughness. However, crack propagation requires longer mechanical tests and cuticle desiccates quickly, becoming brittle during experiments, which greatly alters crack propagation. In this study we tested the effects of various preparation techniques, which preserve hydration for crack propagation testing. Fresh, frozen and petroleum jelly coated adult locust (*Schistocerca gregaria*) tibiae were subjected to 3-point bend tests to detect the effect on the flexural modulus, and establish a best practice sample preparation method for crack propagation.

A11.58 EFFECT OF THE TAIL WING ARRANGEMENT OF RIBBON HALFBEAK ON ITS FLIGHT PERFORMANCE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

YOSHINOBU INADA (TOKAI UNIVERSITY, JAPAN), TAKUMI MATSUDA (TOKAI UNIVERSITY, JAPAN), YUNA TSUCHIYA (TOKAI UNIVERSITY, JAPAN), JUNJI YONEZAWA (TOKYO METROPOLITAN GOVERNMENT, JAPAN)

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Ribbon halfbeak *Euleptorhamphus viridis* is a species of fish capable of flying over sea surface just like flying fish. It has large pectoral fins used as a main wing but lacks large pelvic fins like flying fish have for a horizontal tail. This fish copes with this problem by twisting its rear-body by 90 degrees and uses a dorsal and an anal fins together as a horizontal tail. The horizontal tail needs to generate lift to make balance with the main wing but its performance will be degraded because of the downwash of the main wing. The position of the horizontal tail, therefore, seemed to be important to resolve this problem. In this study, the effect of vertical position of the horizontal tail on the flight performance of the fish was investigated by conducting wind tunnel tests using fish models with different horizontal tail height. In consequence, the lift to drag ratio took the maximum when the horizontal tail located higher than the main wing, indicating the avoidance of downwash realized high flight performance. This result can explain why the ribbon halfbeak in nature flies with its rear-body keeping upper than the pectoral fins.

A11.59 IS THERE OSCILLATION OF GROUND REACTION FORCE DURING WALK OF SMALL ANTS?

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Insects use six legs with characteristic gait pattern for walking. It is well known that there is the vertical oscillation of the center of gravity according to the transition of legs during walking in many insect species, as well as other walking animals. Then, because the oscillation results in energy consumption, it is thought that less oscillation leads to more effective walking.

Here we evaluate the vertical oscillation during the walking of small ants using a Micro Electro Mechanical Systems (MEMS) force plate. The force plate was designed to directly measure the total ground reaction force in vertical direction of an ant (*Messor aciculatus*), whose mass and length are approximately 1.5mg and 4mm, respectively. The fabricated force plate achieved a force resolution of less than 0.5 μ N (thirtieth part of the body weight). Moreover, the plate size is 15mm \times 7mm so that the ant is able to walk for several steps on the plate. From the measurement results, the oscillation of the ground reaction force was not detected during walking across the plate at normal speed with tripod gait. On the other hand, when the walking speed increased, the measured ground reaction force oscillated around the body weight with approximately 25% of the body weight. The results indicate that the amplitude of the oscillation of the ground reaction force during walk of small ants varies according to the walking speed.

A11.60 HYDRODYNAMIC IMPLICATIONS OF THE LONG-NECK IN PLESIOSAURS TESTED USING COMPUTATIONAL FLUID DYNAMICS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

PERNILLE V TROELSEN (LIVERPOOL JOHN MOORES UNIVERSITY, UNITED KINGDOM), DAVID M WILKINSON (UNIVERSITY OF LINCOLN, UNITED KINGDOM), CARLO MELORO (LIVERPOOL JOHN MOORES UNIVERSITY, UNITED KINGDOM), PETER L FALKINGHAM (LIVERPOOL JOHN MOORES UNIVERSITY, UNITED KINGDOM)

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Plesiosaurs are iconic extinct marine reptiles living at the time of the dinosaurs (203-66 Mya), exhibiting some of the longest necks among vertebrates. An understanding of why plesiosaurs had long protruding necks is still lacking. Various hypotheses have been proposed regarding the use of the long neck, primarily concerning feeding strategies. These include snapping at fast moving fish, or extending the feeding envelope from a relatively static position, either floating at the surface or lying immobile at the bottom.

However, the biomechanical implications of such a long neck on fundamental functions such as steady-state locomotion remain untested. We used computational fluid dynamics to explore the hydrodynamic effects of the long neck when bent, simulating both straight and curved necks, during forward locomotion. The analysis included a series of simulations of an idealised three-dimensional (3D) plesiosaur model carried out at different degrees and locations of bending. Drag and flow passing over the 3D model were measured and visualized. Results show a higher drag as degree of bending is increased, as would be expected. A highly curved neck creates a more complex flow pattern including zones of low velocity behind the neck and to the side of the body. We hope that by building on this study we can help to shed light on the biomechanical implications of the long neck in this group of marine reptiles, and more broadly inform hypotheses concerning the lifestyle and evolutionary history of plesiosaurs.

A11.61 BIOMECHANICS AND MATE SELECTION IN THE COPULATORY FLIGHT OF THE BLUE-TAILED DAMSELFLY (*ISCHNURA ELEGANS*)

📅 **WEDNESDAY 5 JULY, 2017** **POSTER SESSION**

👤 HILLA DAVIDOVICH (TEL AVIV UNIVERSITY, ISRAEL), GAL RIBAK (TEL AVIV UNIVERSITY, ISRAEL)

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Blue-tailed damselflies have a unique mating behaviour in which the pair can remain in the copulatory posture for several hours while flying connected from one place to the next. We have shown that during the copulatory flight both sexes flap their wings, but while males increase their aerodynamic effort the females reduce their investment compared to solitary flight. Such intersexual differences in the allocation of aerodynamic power to the joint flight effort can lead to mate selection constraints, due to the fact that the blue-tailed damselflies females are typically larger (~1.2 heavier) than males. To evaluate such constraint we measured the ability of males in different sizes to fly with added loads using the asymptotic load lifting experiment. We found that during voluntary flight blue-tailed males can fly with an added weight of at least 116% (SD 35%, n=21) their body mass. Some mid-size males were capable of flying with an added load equivalent to 170% of their body mass. We found no increase in lifting capability in larger males. To fly with added loads males increased their flapping frequency beyond the frequency observed during solitary flight, but the flapping frequency was not significantly higher than during copulatory flight. These results, and an analysis of the flapping kinematics, suggest that while males are capable of lifting the added weight of an average female, such flight would be close to their maximum capability. Therefore, the assistance from the flapping female is crucial for more demanding maneuvering and climbing flight.

A11.62 GRABBING EVOLUTION BY THE THROAT: FUNCTIONAL REGIONALISATION OF THE AVIAN CERVICAL COLUMN

📅 **WEDNESDAY 5 JULY, 2017** **POSTER SESSION**

👤 RYAN D MAREK (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM), KARL T BATES (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM), PETER L FALKINGHAM (LIVERPOOL JOHN MOORES UNIVERSITY, UNITED KINGDOM)

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Birds have evolved powerful forelimbs that are used in flight. This has rendered these appendages less useful for environmental manipulation than in many of their dinosaurian antecedents. Reliance on the head/neck for feeding and environmental interaction places high selective pressure on cervical form-function potentially explaining the diversity in avian neck morphology. To-date there has been no systematic study of morphological diversity in the avian neck and its correlation with feeding habits. We use a combination of three-dimensional geometric morphometrics (GMM) and qualitative character coding to assess regionalisation within the cervical column of a variety of extant birds. These species represent a diverse array of feeding and functional ecology, cervical count (12-17) and body size. Results provide strong support for 5 cervical subregions (axis, anterior, middle, midposterior, posterior) in all species. The atlas subregion appears to show the strongest signal, with the axis being clearly separate in all studied birds, possibly owing to its function into head stabilisation. Other subregions with a stable cervical count (anterior and posterior) also display a clear functional role. The remaining 2 regions (middle, midposterior) show much variability in cervical count between species (middle 2-6 cervicals, midposterior 1-4 cervicals). These results suggest that whilst the underlying Hox genetics may restrict avians to 5 cervical subregions, expansive variability in the middle and midposterior regions allow the cervical columns of birds to adapt to many different functional ecologies, and may be responsible for the large variety of neck morphologies observed in extant Aves.

A11.63 THE EFFECT OF A BUOYANCY JACKET ON THE HEART RATE OF SWIMMING DOGS

📅 **WEDNESDAY 5 JULY, 2017** **POSTER SESSION**

👤 ALISON P WILLS (HARTPURY UNIVERSITY CENTRE, UNITED KINGDOM), OLIVIA MEDCALF (HARTPURY UNIVERSITY CENTRE, UNITED KINGDOM)

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Canine hydrotherapy is becoming increasingly popular for rehabilitation and as a fitness training aid. During swimming, the use of buoyancy jackets is recommended, particularly for weak or vulnerable patients. There is minimal research investigating the effect of buoyancy aids on physiological parameters in swimming dogs. In this study, seven healthy acclimatised dogs completed six laps of a hydrotherapy pool with and without a buoyancy jacket. When not wearing a buoyancy aid, all dogs were fitted with a

standard safety harness. A heart rate monitor was attached under the buoyancy jacket or harness and kinematic markers were placed on both the dorsal and lateral aspect of the jacket or harness. Data were analysed via a paired t-test to test for differences in displacement and heart rate between the two conditions. Minimum heart rate was significantly lower ($p < 0.05$) in the buoyancy jacket compared to the harness. However, there was no significant difference in maximum ($p = 0.864$) or mean ($p = 0.089$) heart rate between the jacket and harness. Minimum and maximum displacement were significantly higher ($p < 0.001$) for dogs swimming in the buoyancy jacket. Some physiological differences were observed between dogs wearing a buoyancy jacket and harness indicating that further research is warranted to assess how beneficial buoyancy aids are for dogs, particularly those with cardiovascular conditions. When wearing the buoyancy jacket, dogs were positioned higher in the water, which may have energetic implications for swimming and should be considered in the design of future hydrotherapy protocols.

A11.64 CAN FOOT SURFACE AREA IN VIVO PREDICT SKELETAL SURFACE AREA?

WEDNESDAY 5 JULY, 2017 POSTER SESSION

ELEANOR C STRICKSON (LIVERPOOL JOHN MOORES UNIVERSITY, UNITED KINGDOM), JOHN R HUTCHINSON (THE ROYAL VETERINARY COLLEGE, UNITED KINGDOM), PETER L FALKINGHAM (LIVERPOOL JOHN MOORES UNIVERSITY, UNITED KINGDOM)

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The surface area of feet in contact with the ground is a key aspect of an animal's locomotion. Dependent upon the size and shape of this area are underfoot pressures (and consequently forces experienced by the foot), and stability of an animal during locomotion. When studying locomotion of extinct taxa, information can be found in both trackways (recorded in vivo) and skeletons. However, there is a disconnect in many cases between these sources of information - tracks are produced by the soft tissues surrounding the bones, not the bones themselves. We set out to examine whether the area of a skeletal foot could predict in vivo soft-tissue foot surface area. Computed Tomography (CT) scans of several extant tetrapods (covering mammals, crocodylians, birds and salamanders) were used to produce models of the full extent of both the flesh and the bones of their feet. Models were aligned to the horizontal, approximating life positions, and their outlines projected onto a surface to produce two-dimensional 'footprints'. Areas of these projections were calculated using both convex hulls and alpha shapes, to investigate the best method for consistently calculating area. Convex hulls were highly sensitive to pose (e.g. interdigital angles), while alpha-hulling produced more consistent 'tight-fitting' outlines for area calculation. Under-foot area calculated from osteology alone was generally around 50 to 60% that of the area calculated from a fully-fleshed foot. Primary exceptions to this trend were found in horses (as might be expected) and among crocodylians.

A11.65 ON THE RELATIONSHIP BETWEEN INDENTATION HARDNESS AND MODULUS, AND THE DAMAGE RESISTANCE OF BIOLOGICAL MATERIALS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

DAVID LABONTE (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), ANNE-KRISTIN LENZ (UNIVERSITY OF APPLIED SCIENCES BREMEN, GERMANY), MICHELLE L OYEN (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM)

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The remarkable mechanical performance of biological materials is based on intricate structure-function relationships. Nanoindentation has become the primary tool for characterising biological materials, as it allows to relate structural changes to variations in mechanical properties on small scales. However, the respective theoretical background and associated interpretation of the parameters measured via indentation derives largely from research on 'traditional' engineering materials such as metals or ceramics. Here, we discuss the functional relevance of indentation hardness in biological materials by presenting a meta-analysis of its relationship with indentation modulus. Across seven orders of magnitude, indentation hardness was directly proportional to indentation modulus. Using a lumped parameter model to deconvolute indentation hardness into components arising from reversible and irreversible deformation, we establish criteria which allow to interpret differences in indentation hardness across or within biological materials. The ratio between hardness and modulus arises as a key parameter, which is related to the ratio between irreversible and reversible deformation during indentation, the material's yield strength, and the resistance to irreversible deformation, a material property which represents the energy required to create a unit volume of purely irreversible deformation. Indentation hardness generally increases upon material dehydration, however to a larger extent than expected from accompanying changes in indentation modulus, indicating that water acts as a 'plasticiser'. A detailed discussion of the role of indentation hardness, modulus and toughness in damage control during sharp or blunt indentation yields comprehensive guidelines for a performance-based ranking of biological materials, and suggests that quasi-plastic deformation is a frequent yet poorly understood damage mode, highlighting an important area of future research.

A11.66 COMPILING THREE-DIMENSIONAL GEOMETRIES OF LARGE AS WELL AS TINY OBJECTS USING PHOTOGRAMMETRY

WEDNESDAY 5 JULY, 2017 POSTER SESSION

MICHAEL BENNEMANN (WESTPHALIAN INSTITUTE FOR BIOMIMETICS, GERMANY), TOBIAS SEIDL (WESTPHALIAN INSTITUTE FOR BIOMIMETICS, GERMANY)

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For detailed analyses often three-dimensional geometries of specimen were needed. Until now these data could only be acquired through rather costly tomography or by rather tedious sectioning of the specimen into slices. The technique of photogrammetry makes it possible to compile three-dimensional geometries of large as well as tiny objects using standard and affordable equipment and allows to do so without destroying the specimen. First, the specimen is photographed from several perspectives. Simple specimen require approximately 24 images taken in three different heights-levels of the specimen, all adjusted to the centre of the specimen and equally spaced to each other. For more complex specimen more images were needed. Secondly, these images can be joined to compile a three-dimensional geometry using photogrammetry software. The technique of image acquisition is not limited to standard photography, but also suitable for example scanning electron microscopy allowing the compilation of really tiny three-dimensional geometries. In conclusion, photogrammetry is a fast, easy and cost neutral way to compile three-dimensional geometries of large and tiny specimen.

A11.67 GEOMETRY OF DRAGONFLY WING SECTIONS - INFLUENCE ON LIFT AND DRAG

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Wing sections of dragonflies exhibit corrugated profiles. Previous investigations reveal for Reynolds numbers up to $Re=16000$ and an incidence of 3° that the lift to drag ratios of a dragonfly wing section are superior to those of an ordinary NACA0012 profile. This work intends to contribute to the question: "Which influence does the geometry of dragonfly wing sections exert on lift and drag". The wing section is abstracted by a sequence of 9 line elements. The coordinates of the line endings in direction of chord line are calculated by a geometric series and perpendicular to chord line by a symmetrical 4-digit NACA profile. Geometry parameters are the slope of the first line element and the expansion factor r . For $r=1$ the projection lengths to chord line of all line elements are equal, for $r>1$ the projection length of elements increases from leading to trailing edge. Results from computational fluid dynamics for

a profile thickness of 12%, $Re=4000$ and an incidence of 3° show: In comparison to a positive slope a negative slope of the first line element leads to a reduction in drag. With increasing r , the oscillating behaviour of lift and drag reduces. From an aerodynamic view a negative slope of the first element and $r>1$ seems to be favourable. In general, literature data indicate a higher number of shorter line elements near the leading edge in comparison to the rear part. Both, positive and negative slopes of first line elements can be found in literature.

A11.68 RECONSTRUCTING MOVING MORPHOLOGY USING RASPBERRYPI (PIROMM): RANGE OF MOTION IN OSTRICH CERVICAL VERTEBRAE AT PROGRESSIVE STAGES OF DISSECTION

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Advances in recording three-dimensional (3D) moving morphology, such as XROMM, have enabled an unprecedented view of how internal morphology moves, even when hidden by soft-tissue. While such methods offer exceptional levels of accuracy and precision, they are typically associated with costs (both monetary and infrastructure) beyond the means of most researchers, especially students. We present an experimental set-up consisting of three networked raspberry Pi microcomputers with camera modules, in which we recorded the range of motion in ostrich cervical vertebrae at progressive levels of dissection. Pins were inserted through the soft tissue and into the bone, and the neck was manipulated by hand. Camera positions were calibrated using XMA Lab, software developed for the XROMM workflow. The visible heads of the pins were tracked in all three cameras. After dissection, the cervical vertebrae were digitized (with pins still in place) using photogrammetry, and matched to the tracked positions of the pin heads, providing a complete 3D animated scene of the neck in motion. Limitations of the camera module hardware meant that high resolutions and short exposure times were difficult, making the current hardware unsuitable for moderate to fast motion. However, already more advanced camera modules are available at low cost, and we see this system (or systems like it) becoming much more effective in the immediate future. With this system, we were able to reconstruct 3D bone motions with promising levels of accuracy and precision at a very low cost, and this method may be of use to researchers with limited resources.

A11.69 AQUATIC RIGHTING PERFORMANCE OF CHINESE MITTEN CRABS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The biomechanics of the aerial righting has been widely investigated on flightless animals. In contrast to that, the underwater righting has not been explored so far but looks promising. Turning strategies of Chinese mitten crabs (*Eriocheir sinensis*) were investigated to get an overview of the used strategies and the turning performance. For the turning manoeuvre from a dorsal to a ventral position, mostly the fifth pereopod pair is used.

The hydrodynamic forces of these pereopods were measured in experiments by particle image velocimetry (dPIV) and a kinematic approach. Furthermore the crab turning manoeuvre was modelled in a multibody simulation software (ADAMS, rel. 2013.1) in order to compare the necessary turning forces with a theoretical model.

For the initiating stroke of the pereopods, we got by the dPIV measurements mean forces of 0.012 ± 0.0054 N ($n = 9$) and by the kinematic approach a mean force of 0.018 ± 0.004 N ($n = 28$). The simulation results suggest that a constant force of 0.009 N acting for 0.2 s is necessary to perform a 180°-turn.

Measured forces in experiments are about 2 times higher than in the simulation, but in consideration of the duration and the force curve, they lead to a similar momentum and a comparable 180° turn. By adding a crab inspired pereopod to underwater robotics systems (e.g. a ROV platform), they should have the capability to stabilize themselves quick and efficient without the need to add multiple thrusters.

A11.70 DO FLYING FISH HOLD BIOMIMETIC POTENTIAL FOR WING IN GROUND EFFECT CRAFTS?

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Looking up to the sky seems reasonable when looking for inspiration from animals that fly. But one rather exotic animal is easily overlooked: flying fish (Exocoetidae). Due to their low flight height their aerodynamics are influenced by the ground effect - A effect occurring when the ratio of an object's wing span S and flying height h is smaller than 0.5. This ratio is denoted as dimensionless height r . Nevertheless, their flight characteristics have been barely

studied. In this study, a morphometric analysis of flying fish has been performed. Using the morphometric data in a scale up process, a Micro Air Vehicle operating at a Reynolds number of $1 \cdot 10^6$ is derived. The model is analyzed at five values of r , ranging from $r = 5$ to $r = 0.24$, using computational fluid dynamics. Morphometric analysis reveal deviations from literature for the pectoral fin in angle of attack and plan form. When lift and drag coefficients for $r = 0.24$ are compared to $r = 0.5$, the lift coefficient increases by 24.5% for the pectoral fin and drag coefficient decreases by 1.56% due to flight in ground effect.

A11.71 AERODYNAMICS OF MANOEUVRING FLIGHT IN BATS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Research on animal aerodynamics to date has been largely limited to steady level forward flight. In recent years the techniques used in aerodynamic research has developed and the resolution of the aerodynamic tracks we are able to record and reconstruct has been greatly improved - both temporally and spatially. Therefore, it is now possible to analyse how animals execute manoeuvres through differences in timing and magnitude of forces generated by the two wings dynamically through the wingbeat.

In the daily life of any flying animal, manoeuvring is something that is ever present; predators pursuing prey, prey avoiding predator, coping with gusty winds, flying through cluttered environment, and so on. For bats catching insect prey on the wing, it is central and therefore the way they perform their manoeuvres is of direct importance to their biology and ecology.

Here we present the results from the first ever study to explicitly explore the aerodynamics of manoeuvring flight in animals. We performed a set of experiments on Brown long-eared bats (*Plecotus auritus*) flying in a wind tunnel and used time-resolved stereoparticle image velocimetry (PIV) to capture the wakes. We encouraged the bats to perform lateral manoeuvres by laterally translating a thin metal sting holding a mealworm at the instant just before the bat approached it. We identified three main phases for analysis; (i) initiation of the manoeuvre, (ii) execution of the manoeuvre, and (iii) termination of the manoeuvre and stabilization. We discuss the results in the context of flight performance and mechanics.

A11.72 MECHANICAL POWER OF BIRD FLIGHT: CAN BODY ANGLE EXPLAIN A FLAT POWER CURVE?

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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A flying animal accelerates the air around it to generate weight support and thrust. This requires power and according to aerodynamic theory the power should vary in a U-shaped pattern with speed. However, directly measuring the mechanical power has been challenging and indirect measures have been used instead, where some of the previous results have pointed to a flat power curve. Here we estimate the mechanical power directly from tomographic particle image velocimetry measurements of the induced flow in the wake of two pied flycatchers flying in our wind tunnel. The power shows a U-shaped pattern, but the residuals of fitting a standard power equation to the data shows a flatter power curve than expected. Flying birds increase their body angle when flying slowly, which is expected to increase body drag coefficient and the projected body frontal area. When including a variable drag coefficient and frontal area in the model, we get a better fit to our data and speculate that body angle may help explain the flat power curves previously found. Our high resolution measurements also suggest a mechanism for generating a united wake of the two wings, which could help explain the higher span efficiency found in birds compared to bats.

A11.73 JET-SET MOLLUSCS: THE STRUCTURE AND HYDRODYNAMICS OF CUTTLEFISH (*SEPIA OFFICINALIS*) JET PROPULSION SWIMMING

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Rapid and escape locomotion in cephalopods is driven by jet propulsion. Water is taken into the mantle cavity which is compressed by contraction of surrounding circular muscles, expelling water via the siphon propelling the animal. Cuttlefish (*Sepia officinalis*) use jet propulsion swimming for activities ranging from migratory journeys and foraging to interactions with conspecifics and predators. In order to maximise their swimming performance, cuttlefish must effectively transfer energy from their muscles to useful energy in the water. Here, the characteristics of the jets of hatchling and juvenile cuttlefish were measured using particle image velocimetry to track the fluid movements, and the hydrodynamic efficiency was calculated as the ratio of the rate at which work is done against drag to the total power requirements. Two modes of jet propulsion were identified based on the characteristics of the jets: mode I jets consisted of an isolated vortex ring, and mode

II jets in which a leading pinched off vortex ring is followed by a trailing jet of fluid. The propulsive efficiency of animals declined with age, where hatchling animals ($76 \pm 1\%$) were significantly more efficient than juveniles ($61 \pm 2\%$). We also found propulsive efficiency increased at greater swim speeds (ranging from approximately 32 to 91%). Undulatory swimming is considered more efficient than jet propulsion, animals such as chub and rainbow trout, achieve hydrodynamic efficiencies between 70 and 97%, our results suggest cuttlefish are able to achieve comparable hydrodynamic efficiencies through jet propulsion.

A11.74 A QUANTITATIVE AND COMPARATIVE ANALYSIS OF THE WING MUSCLE ARCHITECTURE OF BIRDS OF PREY

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Flight is a key feature in the evolution of birds. Wing anatomy and morphology reflect many aspects of avian biology such as flight capacity. However little is known about the flight musculature, in particular, of the most distal muscles. Therefore, the aim of this work is to investigate the form-function relationship of the wing musculature of birds to understand the role of individual muscles during flight. Dissections of different species of birds of prey were performed to collect numerical data of muscle architecture, which is the primary determinant of muscle function and force-generation capacity. Birds of prey are a highly diverse group that presents different flight styles throughout the taxa, which make them a good model for the purpose of this study. Wing muscle mass (MM) scaled with body mass 1.035 and muscle length (ML) with $MM^{0.343}$, although shoulder and hand musculature significantly differed in comparison with the other regions of the wing. In contrast, fascicle length (FL) scaled to $MM^{0.285}$. Muscle architecture parameters between species are illustrated where we observe different patterns of variation. A proximal-to-distal reduction of MM and ML occurs whereas tendon mass (TM) and insertion tendon length (TL) increases. This arrangement minimizes the wing moment of inertia during flight whilst allowing control of the distal wing. This study presents quantitative information of muscle architecture of birds of prey that helps to understand the functional capacity of the forelimb muscles, which can be used for biomechanical analysis of flight.

A11.75 DO BONY TUBERCLE INSIDE THE FALCON NOSTRIL EFFECT BREATHING DURING HIGH SPEED DIVING?

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

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During prey attacks, falcons reach speed values of more than 300 km/h, indicated as the top speed in animal kingdom. These top speed dives request at least a high manoeuvrability and precision, which results in a high need of energy resp. oxygene for the muscles and the sensory system. To realize a sufficient oxygene supply, continuous breathing during the dives is important for the birds, even against high back pressure in their internal ventilation system. To assist the airflow during breathing, a small bony tubercle inside the nostril of the falcon is discussed but not proved so far.

To test the fluid dynamic effects of this bony structure, a series of 3D models based on MicroCT scans of an adult female falcon (*Falco peregrinus*) nose resp. of the first chamber of the conchae system were produced. The nostril geometry depending nasal airflow was tested via dynamic pressure measurements in a wind tunnel system as well as via computational fluid dynamics analyses. First results show a baffle-like function of the structure, which may offer inspiration for technical airflow systems like air condition and ventilation plants.

A11.76 THREE-DIMENSIONAL MUSCULOSKELETAL MODEL OF THE TIGER SALAMANDER (*AMBYSTOMA TIGRINUM*) FORELIMB DURING TERRESTRIAL LOCOMOTION

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

👤 SANDY M KAWANO (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), JOHN R HUTCHINSON (ROYAL VETERINARY COLLEGE, UNITED KINGDOM)

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Locomotion places some of the greatest loads upon the musculoskeletal system for body support and propulsion, and the application of computational models has provided new insights into how different musculoskeletal designs drive diverse locomotor behaviours. Living salamanders often represent modern analogues to early stem tetrapods and are important functional models to evaluate the locomotor capabilities of a generalised tetrapod bauplan. A computational model of the forelimb in the adult, terrestrial tiger salamander (*Ambystoma tigrinum*) was built to simulate limb mechanics during the lateral sequence walk and help illuminate aspects of the water-land transition. The components of this interactive 3D musculoskeletal model included bones, joints, segment masses, and segment moments of inertia that were obtained from salamander cadavers. 3D models of the bones and muscles were segmented using iodine potassium iodide-enhanced micro-CT scanning. Mass segments used to estimate the inertial properties of the body, arm, forearm, and manus were modelled as geometric primitives of uniform density, and the geometry and architecture of over 20 muscles spanning the shoulder, elbow, and wrist were incorporated as musculotendon units. Simulated data from the musculoskeletal model are compared to published empirical data collected on the inverse dynamics of *A. tigrinum* during terrestrial locomotion to assess how well the predicted data from the model matched biological data. Extension of this musculoskeletal model to the salamander hind limb are ongoing and will provide a more in-depth analysis of the mechanisms underlying differences in locomotor function between the forelimbs and hind limbs.

A11.77 SPOILER-LEGS HELP STREAM MAYFLY LARVAE TO STAY ON THE GROUND

TUESDAY 4 JULY, 2017 POSTER SESSION

• PETRA DITSCHKE (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES), FLORIAN HOFFMANN (UNIVERSITY OF APPLIED SCIENCE BREMEN, GERMANY), SARAH KAEHLERT (ZOOLOGICAL INSTITUTE OF THE UNIVERSITY OF KIEL, GERMANY), ANTONIA KESEL (UNIVERSITY OF APPLIED SCIENCE BREMEN, GERMANY), STANISLAV GORB (ZOOLOGICAL INSTITUTE OF THE UNIVERSITY OF KIEL, GERMANY)

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While a life in running water guarantees a constant supply with food, nutrients and oxygen, the physical features of this current shaped environment can be challenging. Stream insects have developed both, behavioural and morphological strategies to deal with flow forces. The mayfly larva *Ecdyonurus sp.* crawls to current exposed places to graze on algae on top of stones. Their dorso-ventrally flattened body shape is often considered to be an adaptation to flow forces. However, while this body shape considerably reduces drag, it increases lift on the other hand. The latter puts the larvae in danger of being detached from the bottom substrate. We used microscopic techniques, 3D-printing, and force measurements in a wind tunnel to investigate the role of the widened femora of *Ecdyonurus sp.* larvae in counterbalancing these lift forces. Our results show that the larvae use their femora like spoilers generating a downwards directed force (negative lift), which helps them to stay on the ground. The larva can actively regulate the amount of negative lift by changing the femur's angle of attack. Depending on the specific conditions this stream insects can even use the ground effect to support the generation of negative lift. Our results proof that morphological flow adaptations of stream insects can reach far beyond the effects of the overall body shape.

A11.78 TROUT SWIMMING: THRUST AND EFFICIENCY FROM A NUMERICAL PERSPECTIVE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

• ALJOSCHA SANDER (CITY UNIVERSITY OF APPLIED SCIENCES BREMEN, GERMANY), EIZE STAMHUIS (UNIVERSITY OF GRONINGEN, NETHERLANDS), ALBERT BAARS (CITY UNIVERSITY OF APPLIED SCIENCES BREMEN, GERMANY)

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Fish swimming has long been in the scope of experimental biologists. Nevertheless, it has always been a challenge to investigate the mechanical efficiency of fish swimming. In this study, a trout has been abstracted by a two dimensional NACA 0012 profile. The flow around the subcarangiform swimming fish model at a Reynolds number of 10^5 was investigated using computational fluid dynamics. The tail beat frequencies were adapted to typical Strouhal numbers of $St=0.25$ and 0.3 . The flow topology shows attached flow along the body and an inverse Karman vortex street in the wake. For $St=0.25$ an average thrust coefficient of $c_T=0.5$ with an average mechanical efficiency of $\eta=0.48$ was achieved. For $St=0.3$ values of $c_T=0.66$ and $\eta=0.52$ arise. The increase in thrust coefficient with St seems to be reasonable and is in accordance with literature. Lighthill's Elongated Body Theory (EBT) in the improved version from Borazjani und Sotiropoulos delivers efficiencies of 0.7 and 0.69 , respectively. The deviations may result from the two-dimensional numerical simulation of the flow and numerical diffusion. Due to simplified assumptions of EBT, this theory leads to an overestimation of efficiencies. To investigate this gap, three dimensional fish models should be considered in the future. Furthermore, the range of St should be enlarged to receive information about maximum values of η .

A12 OPEN ANIMAL BIOLOGY

ORGANISED BY: CRAIG FRANKLIN (ANIMAL SECTION CHAIR, SEB)

A12.1 PHARMACOLOGICAL CHARACTERISATION OF A VASOPRESSIN/OXYTOCIN-TYPE RECEPTOR IN AN ECHINODERM

WEDNESDAY 5 JULY, 2017 09:00

ESTHER A ODEKUNLE (QUEEN MARY UNIVERSITY OF LONDON SCHOOL OF BIOLOGICAL AND CHEMICAL SCIENCES, UNITED KINGDOM), CHRISTIAN W GRUBER (MEDICAL UNIVERSITY OF VIENNA CENTER FOR PHYSIOLOGY AND PHARMACOLOGY, AUSTRIA), MAURICE R ELPHICK (QUEEN MARY UNIVERSITY OF LONDON SCHOOL OF BIOLOGICAL AND CHEMICAL SCIENCES, UNITED KINGDOM)

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Vasopressin/Oxytocin (VP/OT)-type peptides are a bilaterian neuropeptide family that exert effects via co-evolved receptors and are involved in regulation of diuresis, reproduction and social behaviour in mammals. Recently, we identified a VP/OT-type peptide (asterotocin; CLVQDCPEG-NH₂) that triggers cardiac stomach relaxation (in vitro) and eversion (in vivo) in the starfish *Asterias rubens* (Phylum Echinodermata) (Odekunle et al., unpublished data).

Here we have identified the asterotocin receptor (AstR), which is activated by asterotocin in a concentration-dependent manner when it is expressed in CHO cells ($EC_{50} = 4 \times 10^{-8} M$). To investigate the structure-activity relationships of asterotocin as a ligand for AstR, we tested an asterotocin analog, Arg-vasopressin and a crinoid VP/OT-type peptide (crinotocin). Substitution of D-glutamate at position 8 of asterotocin resulted in a decrease in potency ($EC_{50} = 1 \times 10^{-6} M$), whilst Arg-vasopressin exhibited little or no agonist activity. Crinotocin exhibited very weak agonist activity but at 100 μM decreased the potency of asterotocin by more than 30-fold, suggesting that it can act as a competitive antagonist.

To screen more widely for AstR antagonists a library of known human VP/OTR antagonists and VP/OT-type peptide analogs were tested, including the non-peptide human V_{1a}R antagonist relcovaptan. However, none of these compounds had a significant effect on the activation of AstR by asterotocin. We also designed an asterotocin analog (MCA-Tyr(Me)₂-asterotocin) based on the Manning compound, which is a highly-potent V_{1a}R/OTR antagonist. MCA-Tyr(Me)₂-asterotocin exhibited very weak agonist activity but did not have a significant effect on the potency of asterotocin.

A12.2 INFLUENCE OF THYROID HORMONES ON DEVELOPMENT OF ENDOTHERMY AND VENTILATION IN ALTRICIAL AND PRECOICIAL BIRDS

WEDNESDAY 5 JULY, 2017 09:15

EDWARD M DZIALOWSKI (UNIVERSITY OF NORTH TEXAS, UNITED STATES), TUSHAR S SIRSAT (UNIVERSITY OF NORTH TEXAS, UNITED STATES), SARAH KG SIRSAT (SUNY POTSDAM, UNITED STATES) KINGDOM, CHRISTIAN W GRUBER (MEDICAL UNIVERSITY OF VIENNA CENTER FOR PHYSIOLOGY AND PHARMACOLOGY, AUSTRIA), MAURICE R ELPHICK (QUEEN MARY UNIVERSITY OF LONDON SCHOOL OF BIOLOGICAL AND CHEMICAL SCIENCES, UNITED KINGDOM)

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Thyroid hormones (TH) are key regulators of avian metabolism and are thought to regulate development of endothermy. To better understand the role of TH in avian metabolic development, we examined development of systemic oxygen consumption (V_{O_2}) and ventilation (frequency and tidal volume - V_T) in precocial Pekin ducks and altricial Red-winged Blackbirds (RWBB) under hypothyroid conditions via administration of the thyroperoxidase inhibitor methimazole (87.6 mg/kg). Ducks were dosed on day 24 of a 28-day incubation period and studied during external pipping and 1 day post hatching (dph). Blackbirds were dosed on 2, 3, 4, and 5 dph and measured on days 5, 7, and 9 post-hatching. In Pekin duck hatchlings, hypothyroid treatment blunted the endothermic metabolic response when gradually cooled from 32 to 15°C. Hypothyroid 1 dph hatchlings had a lower resting V_{O_2} that did not increase to the same extent as control animals during gradual cooling. Ventilation frequency of all hatchlings increased during cooling while V_T only increased in control animals, resulting in lower minute ventilation in hypothyroid hatchlings. Hypothyroid RWBB nestlings exhibited a delayed maturation of the endothermic metabolic response. As with hypothyroid ducks, V_T of RWBB nestlings was lower than control nestlings and remained constant during cooling. Our data suggest TH plays an active role in systemic development of endothermic metabolic capacity including ontogeny of ventilation in precocial and altricial birds. In neonatal birds, multiple systems develop in concert to produce an endothermic phenotype, but reduced TH can delay maturation of endothermic capacity and alter physiological function.

A12.3 EVALUATION OF D1 AND D2 DOPAMINE RECEPTORS INVOLVEMENT IN THE FINAL STAGES OF REPRODUCTIVE CYCLE IN PIKEPERCH, A TELEOST FISH

WEDNESDAY 5 JULY, 2017 09:30

JENNIFER ROCHE (UNIVERSITY OF LORRAINE UR AFPA, FRANCE), DANIEL ZARSKI (UNIVERSITY OF WARMIA AND MAZURY DEPARTMENT OF LAKE AND RIVER FISHERIES, POLAND), AMINE KHENDEK (UNIVERSITY OF LORRAINE UR AFPA, FRANCE), CORALIE BROQUARD (UNIVERSITY OF LORRAINE UR AFPA, FRANCE), YANNICK LEDORÉ (UNIVERSITY OF LORRAINE UR AFPA, FRANCE), TOMAS POLICAR (UNIVERSITY OF SOUTH BOHEMIA RESEARCH CENTER OF AQUACULTURE AND BIODIVERSITY OF HYDROCENOSSES, CZECH REPUBLIC), PASCAL FONTAINE (UNIVERSITY OF LORRAINE UR AFPA, FRANCE), SYLVAIN MILLA (UNIVERSITY OF LORRAINE UR AFPA, FRANCE)

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In aquaculture, to produce synchronously offspring of high quality, hormonal treatments such as dopamine receptor (DR) antagonists in combination with salmon-GnRH α are usually applied. Unlike DRD2 (DR-D2) antagonist, there is a lack of data about the effects of DR D1 (DR-D1) antagonist and the respective involvements of both DR in the final stages of reproductive cycle in teleosts. To address this question we have made the first attempt to determine the involvement of DR antagonists in mature pikeperch females, a highly valuable commercial species for which the hormonally-controlled reproduction protocol is of urgent need in aquaculture sector. Fish were injected with metoclopramide (DR-D2 antagonist; 4 or 20 mg.kg⁻¹) or SCH23390 (DR-D1 antagonist; 0.8 or 4 mg.kg⁻¹) in combination or not with salmon-GnRH α . Sampling of brain, pituitary, oocytes and blood were performed before injection, 24 h and 48 h after injection. Last sampling was made at ovulation time or 14 days after injection in fish that did not ovulate. The antagonists alone did not induce ovulation or gonadotropic axis activation except if associated with salmon-GnRH α where all females ovulated. Besides, the application of SCH23390 at 4 mg.kg⁻¹ associated with salmon-GnRH α significantly increased testosterone plasmatic concentrations. These preliminary results would suggest that the DR-D1 would be involved in the final stages of reproductive cycle in pikeperch. Supplementary studies namely brain transcriptome analysis and *in vitro* biological tests of brain culture with dopamine are currently running to go further about the mechanisms induced by these dopamine receptors in pikeperch reproduction.

A12.4 GABA_A RECEPTORS AND BEYOND: FROM MAMMALS TO ZEBRAFISH

WEDNESDAY 5 JULY, 2017 09:45

ARIANNA COCCO (UPPSALA UNIVERSITY, SWEDEN), PER-OVE THÖRNQVIST (UPPSALA UNIVERSITY, SWEDEN), SVANTE WINBERG (UPPSALA UNIVERSITY, SWEDEN)

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The γ -aminobutyric acid (GABA) is the major inhibitory transmitter in the adult vertebrate brain. The ionotropic, anion selective type A GABA (GABA_A) receptors are homo- or heteropentamers. The zebrafish (*Danio rerio* Hamilton, 1822) genome comprises at least twenty-two genes encoding for GABA_A receptor subunits, divided in seven subfamilies (α , β , γ , δ , π , ζ , ρ). The combination, distribution and pharmacology of GABA_A receptor subunits have been widely investigated in the brain of rodents. However, such information is surprisingly scarce in non-mammalian species.

The present study presents the gene expression profile of the almost complete panel of the GABA_A receptor subunits in the adult zebrafish brain, five grouped brain areas, and retinas. As for rats and mice the α_1 , β_2 , γ_2 GABA_A receptor subunits were among those expressed to the highest level in the adult zebrafish brain. Zebrafish, as mammals, alternatively splice the γ subunit in at least two variants. The δ subunit was abundantly transcribed mostly in grouped cerebella. This result once more resembled the mammalian GABA_A receptor system, where the δ subunit is mostly a cerebellar one. The present study highlights a tissue specific expression of the α_6 subunits, with one isoform, α_{6a} , being more abundant in the retinas and the other, α_{6b} , in the brains. The GABA_A receptor system of the zebrafish shares similarities with the mammalian one, but also has its own features. More studies are required, especially at the functional protein level.

A12.5 MOLECULAR CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF THE GULF TOADFISH SEROTONIN TRANSPORTER (SERT)

WEDNESDAY 5 JULY, 2017 10:00

MOLLY H B AMADOR (UNIVERSITY OF MIAMI, UNITED STATES), M DANIELLE MCDONALD (UNIVERSITY OF MIAMI, UNITED STATES)

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The serotonin transporter (SERT) functions in the uptake of the neurotransmitter serotonin (5-HT) from the extracellular milieu. SERT is also known as the molecular target of the selective serotonin reuptake inhibitors (SSRIs), which comprise a common group of antidepressants. We have previously demonstrated that SERT mRNA is widely expressed across tissues in the Gulf toadfish, *Opsanus beta*. Building on these data, the current study comprehensively assesses the sequence, mRNA expression, transport kinetics and pharmacological sensitivity, and physiological functions of a teleost SERT. The 2,022-bp toadfish SERT sequence encodes a protein of 673 amino acids, which

shows 83% similarity to zebrafish SERT and groups with SERT of other teleosts in phylogenetic analysis. SERT cRNA expressed in *Xenopus laevis* oocytes has revealed a K_m value of $2.08 \pm 0.45 \mu\text{M}$, similar to previously reported values for zebrafish and human SERT. Consistent with mRNA expression data, all tissues containing SERT transcript accumulate [^3H]-5-HT. Furthermore, acute systemic blockade of SERT by intraperitoneal administration of the SSRI fluoxetine (FLX) produces a dose-dependent increase in plasma 5-HT, indicating effective inhibition of 5-HT uptake from the circulation. As teleosts lack platelets, which are important 5-HT sequestration sites in mammals, the FLX-induced increase in plasma 5-HT suggests that toadfish tissues may normally be responsible for maintaining low 5-HT concentrations in the bloodstream. Ongoing work is investigating the sensitivity of toadfish SERT expressed in *Xenopus oocytes* to FLX and other SSRIs and using FLX treatment to determine the extent of SERT-mediated 5-HT uptake by the tissues.

A12.6 THE INVOLVEMENT OF RHOPR-CRF/DH IN FEEDING AND REPRODUCTION IN THE KISSING BUG, *RHODNIUS PROLIXUS*

WEDNESDAY 5 JULY, 2017 10:15

IAN ORCHARD (UNIVERSITY OF TORONTO MISSISSAUGA, CANADA), SHIRIN MOLLAYEVA (UNIVERSITY OF TORONTO MISSISSAUGA, CANADA), ANGELA LANGE (UNIVERSITY OF TORONTO MISSISSAUGA, CANADA)

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Feeding, growth and development are interrelated processes in the kissing bug, *Rhodnius prolixus*, with blood gorging initiating both short-term and long-term neuroendocrinological events. In *R. prolixus*, a corticotropin-releasing factor-related diuretic hormone (Rhopr-CRF/DH) is released at feeding and stimulates rapid post-feeding diuresis. The G protein-coupled receptor for Rhopr-CRF/DH is present in digestive tissues, including Malpighian tubules, but also is found in the reproductive system. Thus, Rhopr-CRF/DH may have coordinating roles in *R. prolixus*, associated with feeding-related events and reproduction. Immunohistochemistry reveals diminished CRF-like staining in neurosecretory cells (NSCs) of the mesothoracic ganglionic mass (MTGM) immediately following feeding, with partial restocking two hours later. Temporal qPCR analysis of the Rhopr-CRF/DH transcript is consistent with the immunohistochemical findings, with an increase in transcript expression in the MTGM immediately after feeding. Elevating haemolymph Rhopr-CRF/DH titres by injection of Rhopr-CRF/DH prior to feeding resulted in the intake of a significantly smaller blood meal without affecting the rate of short-term diuresis. Adult females injected with Rhopr-CRF/DH produced and laid significantly fewer eggs. *In vitro* oviduct contraction assays illustrate that Rhopr-CRF/DH inhibits the amplitude of contractions of the lateral oviducts, highlighting one potential mechanism via which the hormone diminishes reproductive capacity.

A12.7 STRESS COPING STYLES IN FISH - BEHAVIOURAL CORRELATES, NEUROENDOCRINE AND MOLECULAR MECHANISMS

WEDNESDAY 5 JULY, 2017 11:00

SVANTE WINBERG (UPPSALA UNIVERSITY, SWEDEN), ARSHI MUSTAFA (UPPSALA UNIVERSITY, SWEDEN), GONCALO ANDRÉ (UNIVERSITY OF WESTERN AUSTRALIA, AUSTRALIA), PER-OVE THÖRNQVIST (UPPSALA UNIVERSITY, SWEDEN)

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In teleosts, as in other vertebrates, divergent stress coping styles usually referred to as proactive and reactive have been described. Proactive animals are bold, aggressive, make active attempts to escape or fight stressors whereas reactive individuals are shy, non-aggressive and respond to threats by a passive response. Differences in stress coping style are also reflected in neuroendocrine stress responses and how individuals perceive and react to their environment. Stress coping style is heritable but is also modified by environmental cues, especially social interaction. We are using zebrafish (*Danio rerio*) as a model to study mechanisms controlling stress coping styles. A selective breeding program is performed to generate two strains of zebrafish displaying divergent stress coping styles. Initial breeders (F0) were identified from a population of wild caught zebrafish by behavioural screening. These fish were used to generate the first generation (F1). Thereafter the procedure is repeated in subsequent generations (F2). The behaviour of fish from the F1 generation of these strains was screened throughout development. In an attempt to study the effects of social stress brain tissue was sampled from proactive and shy reactive fish with experience of being either socially dominant or subordinate in a pair. The results show that already in the F1 generation there is a clear divergence in behavioural profiles of selected fish. Stress coping style, as well as social experience, is also reflected in brain expression of genes related to serotonergic, dopaminergic, histaminergic and the opioidergic systems.

A12.8 MELATONIN REGULATES Na^+ HOMEOSTASIS DURING STRESS RESPONSE IN FISH

WEDNESDAY 5 JULY, 2017 11:15

M C SUBHASH PETER (UNIVERSITY OF KERALA, INDIA)

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Cortisol and thyroid hormones (TH) are well known for its vital role in ion regulation in fishes. On the contrary, the role of melatonin in ion transport particularly on Na^+ regulation and its interaction with cortisol and TH have not yet adequately addressed in fish. We, therefore, focused on Na^+/K^+ -ATPase (nka), the key transporter that maintains Na^+ and K^+ gradients across plasma membrane. We examined the direct action of melatonin on nka functions in the osmoregulatory epithelia of air-breathing fish either in stressed and non-stressed conditions. Analysis of mRNA expression pattern of nka α isoforms such as nka α 1a, nka α 1b and nka α 1c in these tissues showed differential regulation. Immunocytochemical

localization of nkain in these tissues further provided evidence that melatonin could modify the pattern of nka immunoreactivity in hypoxia-stressed fish. Likewise, the nka protein abundance also showed varied pattern after melatonin challenge. Collectively, our data indicate that melatonin can regulate Na^+ homeostasis during stress response by its spatial and differential actions on the varied osmoregulatory tissues in air-breathing fish (supported by grants from iCEIB project, UGC-SAPDRSII and UoK).

A12.9 MAKING A HOME AWAY FROM HOME – PLASTIC PHYSIOLOGY, BEHAVIOUR AND HORMONAL PROFILES OF THE INVASIVE CHAMELEON CICHLID *AUSTRALOHEROS FACETUS* IN SOUTHERN PORTUGAL

WEDNESDAY 5 JULY, 2017 11:30

PEDRO M GUERREIRO (CENTRE FOR MARINE SCIENCES, PORTUGAL), FLAVIA BADUY (CENTRE FOR MARINE SCIENCES, PORTUGAL), JOAO L SARAIVA (CENTRE FOR MARINE SCIENCES, PORTUGAL), PETER C HUBBARD (CENTRE FOR MARINE SCIENCES, PORTUGAL), ADELINO V M CANARIO (CENTRE FOR MARINE SCIENCES, PORTUGAL)

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Invasive species are a growing concern for habitats worldwide. The chanchito, *Australoheros facetus*, a neotropical cichlid which displays high tolerance to abiotic factors and marked social behaviours, is currently found in southern Portugal in small streams with striking seasonal variations in hydrological regimes. We studied its endocrine physiology in the context of environmental acclimation, hierarchy formation and reproductive behaviour.

Fish were exposed to a range of temperature and salinities during short and long-term trials simulating seasonal and/or estuarine conditions. CT_{max} and CT_{min} were determined, showing wide thermal tolerance, and the expression of metabolic and stress genes analyzed. Behaviour is highly and rapidly affected by temperature changes, with territorial aggression ensuing within hours above threshold temperatures. Growth and social behavior are abolished above 18ppt. Monogamous pairs establish and defend breeding territories. Social groups were followed at different periods of the year. Dominance indexes and fish size are highly correlated (♀ : $R=0.55$; ♂ : $R=0.84$; $p < 0.05$) and even very small size differences account for social ranking. No correlation to sex, GSI or HSI was found. Significant differences in initial and final 11K-testosterone (11KT) levels occurred among dominant and subordinates males but not in females of different status. Members of the pair perform parental duties during offspring development but males spend more time patrolling while females care for the young. Whether such behaviours are under hormonal/pheromonal control is currently under investigation.

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A12.10 REGULATION OF RED BLOOD CELL PH AND HAEMOGLOBIN- O_2 AFFINITY DURING THE POST-FEEDING ALKALINE TIDE

WEDNESDAY 5 JULY, 2017 13:50

ROD W WILSON (UNIVERSITY OF EXETER, UNITED KINGDOM), ROBERT P ELLIS (UNIVERSITY OF EXETER, UNITED KINGDOM), MAURICIO A URBINA (UNIVERSIDAD DE CONCEPCIÓN, CHILE)

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The feeding-induced alkaline tide (initial rapid rise, then slower recovery of blood pH due to bicarbonate absorption from the stomach which matches gastric acid secretion) is probably the most common acid-base disturbance fish experience, but the least studied. The alkaline tide may aid oxygen uptake at the gills (via a Bohr shift increasing haemoglobin's affinity for oxygen), but may be maladaptive for tissue oxygen delivery during metabolically demanding digestive processes. We investigated the regulation of extracellular and erythrocyte pH and associated characteristics of haemoglobin-oxygen binding following a meal in voluntary feeding (3% ration) freshwater rainbow trout. Fish experienced the largest alkaline tide found to date in any animal (+0.35 pH units and doubling of $[\text{HCO}_3^-]$). Erythrocytes experienced half the initial alkalosis of plasma (1.5-6 h), but red cell alkalosis was maintained well after plasma pH had recovered by 48 hours. A Blood Oxygen Binding System (BOBS) was used to analyse oxygen dissociation curves (and determine P_{50} values) of microlitre whole blood samples taken at different times after feeding, and to compare with control blood with extracellular acid-base chemistry manipulated *in vitro*. The additional roles of postprandial changes in intracellular ammonium and organic phosphates will also be discussed in relation to satisfying oxygen delivery to tissues during the postprandial period when oxygen consumption is initially elevated more than two-fold and takes more than 4 days to recover to pre-feeding levels.

A12.11 PANCREATIC BASE SECRETION COMPENSATES THE ALKALINE TIDE IN PYTHONS

WEDNESDAY 5 JULY, 2017 14:05

LARS HVASS (DEPARTMENT OF BIOSCIENCE AARHUS UNIVERSITY, DENMARK), TOBIAS WANG (DEPARTMENT OF BIOSCIENCE AARHUS UNIVERSITY, DENMARK)

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Digestion causes an 'alkaline tide' where the concentration of bicarbonate ($[\text{HCO}_3^-]$) in plasma and other body fluids increase as a consequence of gastric acid secretion. This alkaline tide is particularly pronounced in ectothermic animals that feed infrequently on large meals, and where a temporal dissociation of gastric pancreatic secretions occurs. To assess the compensatory role of base secretion into the small intestine from the pancreas and gall bladder, we blocked the passage of chyme into the small intestine by surgical ligation of the pyloric sphincter in catheterized pythons (*Python molurus*). We could then measure changes in arterial pH (pH_a) and partial pressure of CO_2 (Pa_{CO_2})

during the initial 18 hours of the postprandial period, and calculate plasma $[\text{HCO}_3^-]$ using the Henderson-Hasselbalch equation.

The un-manipulated control animals exhibited the typical rise in plasma $[\text{HCO}_3^-]$ of approximately 8 mM accompanied by a rise in arterial PaCO_2 that alleviated the effects on pH. The rise of $[\text{HCO}_3^-]$ was clearly exacerbated when passage of the acidic stomach content was prevented from entering the small intestine. Arterial pH, PaCO_2 and plasma $[\text{HCO}_3^-]$ increased approximately 2-, 4- and 3-fold, respectively, compared to control animals. This finding demonstrates that acid-induced bicarbonate secretion into the small intestine indeed is a major mechanism of acid-base balance during digestion and that this mechanism greatly compensates the alkaline tide during the postprandial period in Pythons.

A12.12 DOES THE LEFT AORTA IN CROCODILIANS PROVIDE PROTON-RICH BLOOD TO THE GUT DURING DIGESTION?

WEDNESDAY 5 JULY, 2017 14:20

JUSTIN L CONNER (UNIVERSITY OF NORTH TEXAS, UNITED STATES), JANNA L CROSSLEY (UNIVERSITY OF NORTH TEXAS, UNITED STATES), RUTH ELSEY (ROCKEFELLER WILDLIFE REFUGE, UNITED STATES), DEREK NELSON (UNIVERSITY OF NORTH TEXAS, UNITED STATES), OLIVER H WEARING (MCMASTER UNIVERSITY, CANADA), TOBIAS WANG (AARHUS UNIVERSITY, DENMARK), DANE A CROSSLEY II (UNIVERSITY OF NORTH TEXAS, UNITED STATES)

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Reptiles are endowed with the capacity to differentially perfuse the systemic and pulmonary vascular circuits via autonomic regulation of the heart and the vascular trees. While this aptitude is widely recognized, the role of 'shunting' as a homeostatic mechanism to match convective transport with tissue demand remains questionable. In crocodilians, it has been hypothesized that a pulmonary vascular bypass of systemic venous blood, right to left shunt (R-L), serves to deliver CO_2 -rich blood with protons needed for gastric acid secretion during digestion. This hypothesis is partially based on the unique crocodilian vascular anatomy where a left aorta arises from the right ventricle, and appears to preferentially supply the gastrointestinal system, whereas the right aorta emerges from the left ventricle. However, direct measurements of blood gases in the major systemic outflow vessels and venous return to the heart have not been conducted. For this reason, we measured blood parameters including PO_2 , PCO_2 , pHe, $[\text{HCO}_3^-]$ in both aorta (right and left) as well as both atria following ingestion of a gavage fed standardized meal (5% body mass). Blood samples were withdrawn at 3hr, 6hr, 12hr, 24hr, 36hr, 48hr into the digestive period. At no point did PCO_2 or pH differ between the left and right aorta, whereas PO_2 was significantly lower in the left aorta at several time points during digestion. Our findings fail to support the hypothesized role of the R-L shunt to increase CO_2 delivery to the gastrointestinal system in crocodilians after feeding.

A12.13 CLASS MATTERS: EVOLUTION OF CARBONIC ANHYDRASE IN MARINE FISH

WEDNESDAY 5 JULY, 2017 14:35

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Carbonic anhydrase (CA), an enzyme found in almost all living organisms, catalyses the reversible dehydration/hydration reactions of carbon dioxide (CO_2). In most vertebrates, CA within red blood cells (rbc) plays a critical role in CO_2 transport and excretion across epithelial tissues. Ancestral fish, such as hagfish and elasmobranchs, are unique in that they possess plasma-accessible membrane-bound CA-IV in the gills, allowing CO_2 excretion to occur completely outside of the rbc, and possibly alleviating the requirement for high activity rbc CA. Through homology cloning techniques, we identified the putative protein sequences for rbc CA from 5 shark and 3 ray species, as well as the Pacific hagfish. In all cases, the structure contained a modification of the proton shuttle residue His-64 - an alteration known to dramatically reduce catalytic rate in mammalian isoforms. Bayesian analyses of 54 vertebrate CA isoforms, including the novel hagfish and elasmobranch rbc CA sequences, demonstrate independent events that led to this modification of His-64 and the possible loss of high activity CA in vertebrates. Measurements of total CA activity across select teleosts and elasmobranchs suggest that shark species have significantly reduced rbc activity compared to teleosts. Surprisingly, ray species, which also have the His-64 modification within their active site structure, have comparable total CA activity to teleosts, which may suggest a reduced reliance on plasma-accessible CA-IV for CO_2 excretion.

A12.14 FUNCTIONAL CHARACTERIZATION OF HEMOGLOBIN ISOFORMS FROM HIGH AND LOW ALTITUDE GEESE SPECIES

WEDNESDAY 5 JULY, 2017 14:50

AGNIESZKA JENDROSZEK (AARHUS UNIVERSITY, DENMARK), HANS MALTE (AARHUS UNIVERISTY, DENMARK), ROY E. WEBER (AARHUS UNIVERISTY, DENMARK), JAY F. STORZ (UNIVERSITY OF NEBRASKA, UNITED STATES), ANGELA FAGO (AARHUS UNIVERISTY, DENMARK)

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Each year bar-headed goose (*Anser indicus*) migrate over the Himalaya to overwinter in India. Energetically demanding flap flight at altitudes where the oxygen is reduced to approximately 30% of sea level is an astonishing achievement in part due to an adaptive increase in the oxygen affinity of bar-headed goose hemoglobin (Hb), compared to that of lowland greylag goose (*Anser anser*). Although avian red blood cells contain two Hb isoforms, HbA and HbD, the oxygen affinities of Hb isoforms of bar-headed and greylag goose have not been characterized separately yet. In the present study, we used equilibrium methods to characterize in detail oxygen-binding and allosteric properties of purified HbA and HbD from bar-headed and greylag goose, in presence and absence of the allosteric effector inositol hexaphosphate (IHP), an analogue of the avian red blood cell organic phosphate. Both bar-headed goose Hb isoforms displayed higher intrinsic oxygen affinity than the respective greylag goose Hbs. This difference in Hb oxygen affinity between the species was even more marked with added IHP, which reduced oxygen affinity at least 9-fold. Curve fitting analyses according to the two-state MWC model indicated that the effect of IHP was primarily due to stabilisation of highly labile tense state molecules. Moreover, IHP stabilised low-affinity tense and high-affinity relaxed Hb tetramers which otherwise tended to dissociate to dimers. The present study provides a novel detailed analysis on the allosteric regulation properties of geese isoHbs explaining the high oxygen affinity of bar-headed goose hemoglobins at a molecular level.

A12.19 IONS BEFORE OXYGEN: LARVAL LAMPREYS AND PROTOCHORDATE REPRESENTATIVES QUESTION THE ORIGINS OF CHORDATE GILL FUNCTION

WEDNESDAY 5 JULY, 2017 15:45

MICHAEL A SACKVILLE (UNIVERSITY OF BRITISH COLUMBIA, CANADA), CHRISTOPHER B CAMERON (UNIVERSITY OF MONTREAL, CANADA), JONATHAN M WILSON (WILFRED LAURIER UNIVERSITY, CANADA), COLIN J BRAUNER (UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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The gill is believed to have played a key role in the adaptive radiation of early chordates by supplanting the skin as the dominant site for oxygen uptake. In this untested hypothesis, shifting oxygen uptake to the gill relaxed constraints associated with oxygen uptake at the skin, thus allowing chordates to increase body size, dermal

thickness and activity. Interestingly, larval teleosts undergo similar increases to body size, dermal thickness and activity during development. However, the larval teleost gill first supplants the skin as the dominant site for ion flux well before oxygen uptake. Here, we want to determine if the gill also became critical for ion flux before oxygen during early chordate evolution. We use larval lampreys as a representative system to test how increasing organism size might have affected early chordate gill function. We directly measure the flux of oxygen, carbon dioxide, ammonia and sodium simultaneously at the gill and skin of whole larvae in vivo across a range of body sizes (0.02-2.00 g). We find that sodium flux is dominated by the gill at much smaller body sizes than gas flux, even when challenged by hypoxia and temperature. Complimentary data collected in more ancestral protochordate representatives (amphioxus and acorn worms) also indicate a dominant role for the gill in ion flux before gas. Our results suggest that ion regulation rather than gas exchange might have been the primary function of the early chordate gill, questioning long-standing views of chordate gill origins and the constraints shaping early chordate evolution.

A12.20 INTERACTIVE EFFECTS OF SALINITY AND AMMONIA STRESS ON MARINE FISH: INSIGHTS FROM GENOME-WIDE TRANSCRIPTIONAL ANALYSIS

WEDNESDAY 5 JULY, 2017 16:00

AMIT KUMAR SINHA (UNIVERSITY OF ANTWERP, BELGIUM), MARIOS N NEKTARIS (UNIVERSITY OF ANTWERP, BELGIUM), GAURAV ZINTA (UNIVERSITY OF ANTWERP, BELGIUM), AJAY A KUMAR (UNIVERSITY OF ANTWERP, BELGIUM), GERRIT T S BEEMSTER (UNIVERSITY OF ANTWERP, BELGIUM), RONNY BLUST (UNIVERSITY OF ANTWERP, BELGIUM), GUDRUN DE BOECK (UNIVERSITY OF ANTWERP, BELGIUM)

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The objective of the present study was to elucidate genome-wide transcriptional responses underlying reduced seawater salinity challenge and ammonia toxicity as single and combined factor in European seabass (*Dicentrarchus labrax*). Fish were progressively acclimated to normal seawater (32 ppt) and reduced seawater salinity (10 ppt). Following acclimation to these salinities for two weeks, fish were exposed to high environmental ammonia (HEA, 1.18 mM representing 50% of 96 h LC₅₀) for 12h, 84h and 180h. RNA sequencing (Illumina HiSeq platform) of brain tissue samples from the experimental conditions yielded a total of 12,490 contigs, out of which 2,128 were significantly modulated in any of the applied treatments (salinity, HEA or combination). Low salinity resulted in the up-regulation of 370 and down-regulation of 832 contigs, whereas in HEA exposure 122 contigs were up-regulated and 270 contigs were down-regulated. While looking for the combinatorial effects, we found that 74 contigs were commonly up-regulated and 94 were down-regulated in both these treatments. Gene enrichment analysis revealed that genes differentially expressed in response to salt stress were highly enriched for Na⁺ transporter, cell adhesion and communication, transmembrane signalling receptor, Na⁺/K⁺-ATPase and oxidative stress. Genes which were differentially expressed to HEA showed enrichment in amino acid biosynthetic/metabolic process, anti-oxidant system, neurotransmitter and ion-regulation. In combination treatment, ion transporters, oxidative and anti-oxidant related genes were highly represented. Overall, our data highlights the complexity of transcriptional responses which are triggered when fish face individual and/or combinatorial environmental challenges.

A12.21 EPIDERMAL EPIDEMIC: EFFECTS OF CHYTRIDIOMYCOSIS ON AMPHIBIAN EPITHELIAL TRANSPORT DURING SLOUGHING

WEDNESDAY 5 JULY, 2017 16:15

NICHOLAS C WU (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA), JULIA GAUBERG (YORK UNIVERSITY, CANADA), REBECCA L CRAMP (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA), CRAIG E FRANKLIN (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA)

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Amphibians are at the forefront of the current global biodiversity crisis, with declines and extinctions due in part to the emergence of a novel fungal skin disease, chytridiomycosis. The cause of death has been linked to the disruption of the skin function and low electrolyte levels in the body. Amphibians increase the rate of skin shedding (sloughing) as an attempt to remove cutaneous pathogens before the onset of the disease. However, sloughing also causes physiological changes in the skin function, specifically cutaneous ion transport. Thus, sloughing may act as a double edge sword, where in the process of removing the pathogen, it may exasperate the effects of cutaneous ion loss. We therefore hypothesised that infected frogs would show a reduction or inhibition of epithelial ion transporters, which are responsible for regulating normal skin function and internal electrolyte balance. We also predicted that during sloughing infected frogs would demonstrate an increase in cutaneous ion loss, and greater electrolyte imbalance indicating a potential susceptible period which ultimately leads to hyponatremia. Green tree frogs *Litoria caerulea* were infected with chytrid fungus and sloughing episodes monitored. We measured cutaneous ion loss, blood electrolyte levels, abundance, activity, distribution, and expression of two key ion transport proteins Na^+/K^+ -ATPase (NKA), and the epithelial sodium channel (ENaC). In support of our hypotheses, we found significant changes in osmoregulatory function and epithelial transport in infected frogs during sloughing that impacted upon ionic and osmotic homeostasis.

A12.22 GAP JUNCTIONS PLAY A ROLE IN COUPLING ION FLOW THROUGH THE PRINCIPAL AND SECONDARY CELLS IN THE MALPIGHIAN TUBULES OF LEPIDOPTERANS

WEDNESDAY 5 JULY, 2017 16:30

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The larvae of many species of lepidoptera (butterflies and moths) are major agricultural pests. High rates of feeding and growth and maintenance of extremely alkaline conditions in the anterior midgut pose dramatic ionoregulatory challenges for these insects. Excretion in insects is accomplished by the combined actions of the Malpighian tubules and hindgut, which together form the functional kidney. The fluid secretory portion of the tubule is composed of principal and secondary cells. Na^+/K^+ ATPase, which energizes ion transport

in vertebrate epithelia, has been assumed to play a minor role in this process. Current study, however, found Na^+/K^+ ATPase to be localized to the basolateral membrane of principal cells in the Malpighian tubules of *T. ni*. Interestingly, blocking Na^+/K^+ ATPase activity with ouabain resulted in diminished reabsorption of Na^+ and K^+ by the secondary cells (which lack Na^+/K^+ ATPase immunoreactivity), suggesting coupling of the two cell types via gap junctions. The effects of drugs which block gap junctions indicated coupling of ion transport in the two cell types and the levels of transcripts encoding several gap junction proteins in the Malpighian tubules changed in response to variations in dietary Na^+ and K^+ . The coupling of ion transport between the two cell types is hypothesized to aid recycling of ions necessary for constant fluid secretion and excretion of metabolic wastes in Lepidoptera.

A12.15 THE EFFECTS OF ANAESTHESIA AND SURGERY ON CARDIORESPIRATORY PHYSIOLOGY AND PHYSIOLOGICAL STRESS MARKERS IN THE TERRAPIN *TRACHEMYS SCRIPTA*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

CATHERINE J WILLIAMS (AARHUS UNIVERSITY, DENMARK), WILLIAM JOYCE (AARHUS UNIVERSITY, DENMARK), DANE CROSSLEY II (UNIVERSITY OF NORTH TEXAS, UNITED STATES), MADS F BERTELSEN (COPENHAGEN ZOO, DENMARK), TOBIAS WANG (AARHUS UNIVERSITY, DENMARK)

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Reptiles are increasingly treated in veterinary practice and their use continues in physiological research. However, information on optimal anaesthetic and analgesic protocols for reptile are poorly delineated in comparison with other taxa and the cardiorespiratory effects of common anaesthetic agents are largely unknown. Our overall aim was to provide insight into the impact of anaesthetic and surgical interventions in reptiles, hence, we evaluated whether atropine ($1 \text{ mg} \cdot \text{kg}^{-1}$) alters inhalation anaesthesia induction and recovery in a species of terrapin (*Trachemys scripta*). The cholinergic antagonist atropine abolishes vagally-mediated regulation of the pulmonary artery, resulting in a relative increase in pulmonary blood flow and tachycardia in conscious terrapins. We therefore hypothesised that atropine would hasten recovery from inhaled volatile (isoflurane) anaesthesia via changing cardiac shunting patterns during isoflurane elimination. We quantify the effect of atropine, as well as that of intracoelomic surgery to place cannulae and flow probes, under isoflurane anaesthesia with analgesia (lidocaine, prilocaine and meloxicam) on blood flow patterns, pressure and heart rate over 48 h recovery. We also compared the perioperative effect of isoflurane with that of pentobarbital, the standard anaesthetic for use during in situ studies. Finally, we assess peri and postoperative catecholamine concentrations taken together with plasma glucose, lactate and cardiovascular parameters as physiological stress proxies. Atropine did not significantly affect recovery time when administered preoperatively in animals undergoing instrumentation (recovery time: atropinised animals 114 ± 44 , control 127 ± 56 mins), but reflex assessment revealed a more stable anaesthetic with longer recovery after atropine in non-instrumented animals.

A12.16 TERRESTRIAL ANURAN RANA TEMPORARIA AVOIDS THERMALLY INDUCED HEART FAILURE DUE TO MAINTENANCE OF ATRIOVENTRICULAR AND VENTRICULAR CONDUCTION

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Most of ectothermic animals are unable to cope with acute temperature changes due to heart failure. It has been suggested that heart failure occurs as a result of atrioventricular (AV) or ventricular conduction blockade. However, amphibian anurans are well known poikilotherms and their heart tolerates well the fast temperature changes in wide diapason. Electrophysiological mechanisms underlying the preservation of cardiac performance in anurans are not completely elucidated.

Here, standard surface ECG was recorded in anesthetized frog *Rana temporaria* after double autonomic blockade at various temperatures (2-25°C, n=18) and RR, PQ intervals were calculated. Optical mapping approach was used to evaluate AV and ventricular conduction at various temperatures (4-25°C). Optical action potentials were recorded using the CCD camera (WuTech Instruments) in multicellular isolated, perfused, di-4-ANEPPS-stained and blebbistatin-treated preparations (n=6) consisting of atrial and ventricular heart regions with intact AV ring.

Frogs demonstrated normal ECG in diapason from 2 to 25°C. Both RR and PQ intervals revealed similar temperature dependence, Q10 coefficients. Temperature alteration never caused AV blocks, however, cooling resulted in prolongation of AV delay (210±22 at 25°C and 740±102 ms at 2°C). Identical (associated with interatrial septum ridges) pathways of conduction in frog AV ring were observed at all tested temperatures. Excitation in ventricle was conducted without blocks or disturbances in 4-25°C range. Temperature alteration had significantly weaker influence on ventricular than on AV conduction. Thus, maintenance of AV and ventricular conduction may underlie thermal tolerance of anuran heart. This study is supported by Russian Science Foundation grant 14-15-00268.

A12.17 NEW MECHANISM OF ACTION POTENTIAL WAVEFORM MODULATION BY ADRENERGIC COMPOUNDS IN FISH HEART

WEDNESDAY 5 JULY, 2017 POSTER SESSION

DENIS V ABRAMOCHKIN (LOMONOSOV MOSCOW STATE UNIVERSITY, RUSSIA), THOMAS ELIOT HAWORTH (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), MAEVA GACOIN (UNIVERSITE DE POITIERS, FRANCE), VLADISLAV S KUZMIN (PIROGOV RUSSIAN NATIONAL RESEARCH MEDICAL UNIVERSITY, RUSSIA), HOLLY A SHIELS (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

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Adrenaline has both a positive chronotropic (rate) and inotropic (force) effect on the heart which increases cardiac output and thus participates in the 'fight or flight' response. The inotropic increase in contractile force is driven mainly by an adrenergic increase in myocyte Ca^{2+} influx during cardiac action potentials (APs). In the present study we demonstrate that ventricular AP is prolonged in myocytes from rainbow trout following stimulation of α and β adrenoreceptors with the endogenous ligand, adrenaline (10^{-6} M). Interestingly, selective α -adrenoreceptor stimulation (isoprenaline/isoproterenol, 10^{-6} M) produced a weaker prolongation of AP than selective α -adrenoreceptor stimulation (phenylephrine, 10^{-6} M). While β -adrenoreceptor induced AP prolongation is attributed to enhancement of the L-type Ca^{2+} -current (I_{CaL}), we show that stronger α -mediated prolongation is due to the attenuation of K^+ delayed rectifier current (I_{Kr}) in both atrial and ventricular myocytes. Adrenaline (10^{-6} M) reduced the maximal tail I_{Kr} to 61.4±3.9% of control in ventricular and 76.4±2.7% in atrial myocytes. This effect was reproduced by the α -adrenergic agonist, phenylephrine (10^{-6} M), but not the β -agonist isoproterenol (10^{-6} M), which tended to increase tail I_{Kr} . Interestingly, adrenaline (10^{-6} M) in the presence of β_1 and β_2 -blockers (10^{-6} M atenolol and 10^{-6} MICI118.551, respectively) also inhibited I_{Kr} , slowing the repolarization and prolonging AP. The inward rectifier K^+ current (I_{K1}) was insensitive to the adrenomimetics. Thus, the increase of APD following sympathetic adrenergic stimulation in fish heart is mediated by activation of α -adrenoreceptors leading to I_{Kr} suppression. We speculate this mechanism may complement the well-known β -adrenoreceptor mediated enhancement of I_{CaL} in the inotropic response to adrenaline.

A12.18 FLUORESCENT IMPLANTABLE ELASTOMER TAGS FOR THE MEASUREMENT OF OXYGEN WITHIN INSECTS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Monitoring oxygen partial pressure (PO_2) within an organism provides essential information for understanding oxygen uptake and numerous other aspects of respiratory physiology. However, in vivo measurements using current implantable O_2 probes are limited by the small size of many organisms; This study sought to develop fluorescent implantable elastomer tags (FIETs) as an alternative method for accurate measurement of PO_2 within small semi-transparent organisms. The FIETs were designed with an O_2 -permeable matrix and a fluorescent, ratiometric dye system: the fluorescence of an indicator dye (emission 650nm) is quenched in the presence of O_2 , whereas the fluorescence of a reference dye (emission 530nm) does not change. A custom microfluidic chip produced highly uniform batches of spherical FIETs, with FIET diameters as small as 67 μm . The FIETs response to O_2 was quantified by measuring both dyes' fluorescence intensities across a range of O_2 tensions (0-20.26 kPa) using an inverted fluorescence microscope. Our results show a linear relationship between the fluorescence ratios and PO_2 ($R^2 = 0.963$), although over time photobleaching causes an apparent drift in the PO_2 signal. Next, we will demonstrate the FIETs' use in a biological context and observe whether auto-fluorescence interferes with their accuracy.

A12.23 HIGH COST OF CALCIFICATION AND UNEXPECTED INTRACELLULAR ADAPTATIONS IN MARINE MUSSELS LIVING AT EXTREMELY LOW SALINITY IN THE BALTIC SEA

WEDNESDAY 5 JULY, 2017 POSTER SESSION

TRYSTAN SANDERS (GEOMAR, GERMANY), JÖRN THOMSEN (GEOMAR, GERMANY), JENNIFER NASCIMENTO SCHULZE (GEOMAR, GERMANY), LARA SCHMITTMANN (GEOMAR, GERMANY), CHRISTIAN BOCK (ALFRED WEGENER INSTITUTE, GERMANY), FRANK MELZNER (GEOMAR, GERMANY)

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The steep salinity gradient from 30 psu to 6 psu in the Western Baltic Sea provides an excellent system to investigate adaptation to low salinity in marine calcifying invertebrates. Climatic models predict a desalination of the Baltic Sea over the next 100 years and a shifting of the salinity gradient further west increasing the osmotic stress on marine organisms, such as the blue mussel, already living at the edge of their environmental tolerance range. Metabolomic analysis using 1H -NMR spectroscopy was used to investigate changes in intracellular organic osmolytes and energy budgets from different populations were measured following long-term acclimation. Results reveal that extremely low salinities (6 psu) do not elicit significant alterations in whole animal oxygen consumption;

however the energetic cost of calcification is dramatically increased 2-3 fold. This is mirrored by strongly elevated metabolic rates of mantle tissue, the tissue responsible for shell secretion. Contrary to expectations, intracellular organic osmolyte analysis showed that at the same salinity, low salinity adapted populations maintain lower concentrations of the main osmolytes taurine, glycine and aspartate compared to high salinity adapted populations. Ongoing work focuses on analyzing intracellular inorganic ion concentrations to see if reduced external salinity elicits similar patterns of change as with organic osmolytes. These findings present some potential costs of adapting to life in an extremely dilute environment as well as the ability for marine mussels to drastically change their intracellular environment.

A12.24 TEMPORAL ASSESSMENT OF METABOLIC RATE, AMMONIA DYNAMICS AND ION-STATUS IN COMMON CARP DURING FASTING: OPTIMIZING FASTING PERIOD PRIOR TO TRANSPORT

WEDNESDAY 5 JULY, 2017 POSTER SESSION

JYOTSNA SHRIVASTAVA (UNIVERSITY OF ANTWERP, BELGIUM), AMIT KUMAR SINHA (UNIVERSITY OF ANTWERP, BELGIUM), SARAH CANNAERTS (UNIVERSITY OF ANTWERP, BELGIUM), ELINE VERRSTTE (UNIVERSITY OF ANTWERP, BELGIUM), RONNY BLUST (UNIVERSITY OF ANTWERP, BELGIUM), GUDRUN DE BOECK (UNIVERSITY OF ANTWERP, BELGIUM)

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The objective of this study was to evaluate an ideal fasting period prior to the fish transportation. For this purpose, common carp (*Cyprinus carpio*) were progressively fasted up to 14 days. Temporal effect of fasting on oxygen consumption rate (MO_2), ammonia excretion rate (J_{amm}), plasma ammonia (T_{amm}), plasma ions, branchial Na^+/K^+ -ATPase (NKA) and H^+ -ATPase activity, as well as branchial mRNA expression of NKA, H^+ -ATPase, Na^+/H^+ exchanger (NHE-3) and Rhesus (Rh) glycoproteins were assessed. Results show that MO_2 and J_{amm} were considerably depressed from 4-6 days of fasting onwards. This coincided with the onset of higher resting plasma T_{amm} from day 6 onwards, possibly because in fasted fish the basal expression levels of Rhcg-a and Rhcg-b were only maintained up to 6 and 8 days respectively, after which a down-regulation was recorded. Plasma $[Na^+]$ and $[Cl^-]$ were temporarily reduced during 4-8 days of fasting, while an augmented $[K^+]$ was evident. The transcript level of NHE-3 was raised in 12-14 days fasted fish, which along with up-regulation in Na^+/K^+ -ATPase activity and mRNA expression facilitated the recovery of $[Na^+]$ to control level. First signs of energy store depletion in liver, especially glycogen, were recorded from day 8 onwards. Overall, these data suggest that the beneficial reduction of metabolic rate (MO_2 and J_{amm}) started at 6 days of fasting. Interestingly, at this time fish were still able to regulate ammonia transport efficiently, and did not compromise their energy stores yet. Therefore, we propose 6 days as an ideal fasting period before transport.

A12.25 ONTOGENY OF UREA AND AMMONIA TRANSPORTERS IN MAHI-MAHI (*CORYPHAENA HIPPURUS*) EARLY LIFE STAGES

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The timing and location of the 2010 Deepwater Horizon (DWH) incident within the Gulf of Mexico coincided with the spawning of many ecologically and commercially important fish species, such as mahi. Recent studies by our lab and others revealed upregulation of ammonia transporter genes (Rhag and Rhbg) and increased ammonia excretion in oil-exposed mahi embryos. The present study found that, similar to other teleosts, mahi avoid the toxic build-up of ammonia by being largely ureotelic during the embryonic stage and gradually switch to being ammonotelic at the time of hatch. Thus, any change in the timing of these processes could indicate significant physiological impacts with implications for survival. In this study, unexposed mahi embryos and larvae revealed mRNA expression changes for seven genes involved in nitrogenous waste excretion over the initial 96 h of life: Rhag, Rhbg, Rhcg1 and Rhcg2 (ammonia transporters), SLC14a2 (urea transporter), NHE2 and NHE3 (Na⁺/H⁺ exchanger) which are potentially involved in ammonia transport. The mRNA levels of the transporters are highly consistent with ammonia and urea excretion rates in mahi embryos we observed. Additionally, *in situ* hybridization examined tissue-specific expression of these genes. Each Rh protein has a unique expression pattern along the gills, skin and yolk sac. Contrary to what has been shown for other species, NHEs appear to play less a role in ammonia transport. The results contribute to our understanding of ammonia excretion in pelagic fish and physiological consequences of oil exposures during early development.

A12.26 NITRIC OXIDE INHIBITS NaCl SECRETION ACROSS THE OPERCULAR EPITHELIUM OF THE SEAWATER KILLIFISH BY DUAL MECHANISMS: cGMP SIGNALLING AND S-NITROSATION

WEDNESDAY 5 JULY, 2017 POSTER SESSION

LUCIE GERBER (AARHUS UNIVERSITY, DENMARK), FRANK B JENSEN (UNIVERSITY OF SOUTHERN DENMARK, DENMARK), STEFFEN S MADSEN (UNIVERSITY OF SOUTHERN DENMARK, DENMARK), WILLIAM S MARSHALL (ST. FRANCIS XAVIER UNIVERSITY, CANADA)

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Active transport of ions is essential for the maintenance of homeostasis and is under control of a large array of signalling molecules and mechanisms. The signalling molecule nitric oxide (NO), known to influence various physiological functions, emerges as a novel regulator of epithelial ion transport pathways in vertebrates. Hypotheses for NO modulation of ion transport include (1) binding to soluble guanylyl cyclase (sGC) to stimulate cyclic guanosine monophosphate (cGMP) production and (2) S-nitrosation of ion transporters. However, the precise role of NO in ionic regulation and mechanism(s) triggering its action remain largely unexamined in fish. An Ussing chamber approach was used to explore the involvement of NO in controlling ion transport across the opercular epithelium of the seawater killifish and to test its cGMP-dependence. In parallel, a modified biotin-switch assay was carried out to investigate S-nitrosation of proteins by NO. We found that the epithelial short-circuit current, an indicator of NaCl secretion, was decreased by stimulation of endogenous NO production and addition of exogenous NO. We further demonstrated that this NO-induced decrease was abolished by inhibitors of sGC, revealing cGMP dependence of NO signalling. In addition, we reported S-nitrosation of several proteins by exogenous NO, including the major ion pump: Na⁺/K⁺-ATPase. Our results demonstrate how NO is likely to function as a physiological regulator of ion transport processes in fish via combined mechanisms. This study brings new insight into the paracrine modulating system of fish involved in the fine-tuning of ion transport to deal with fluctuating and challenging environments.

A12.27 THE USE OF PULSATILE UREA EXCRETION TO CHEMICALLY COMMUNICATE REPRODUCTIVE STATUS IN GULF TOADFISH, *OPSANUS BETA*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Gulf toadfish (*Opsanus beta*) are uniquely capable of switching from excreting ammonia as their primary nitrogenous waste to excreting predominantly urea in distinct pulses across the gill. Previous studies suggest that these pulses may be used for intraspecific chemical communication. Many aquatic organisms release sex pheromones, but little is known about the use of pheromones in marine teleosts. To determine if pulsatile urea excretion communicates reproductive status, toadfish were sexed using ultrasound and delivered pre-conditioned seawater (PC-SW) that previously housed a conspecific of the opposite sex, a prey cue made of shrimp homogenate (attraction control), or a conspecific chemical alarm cue (avoidance control). Behaviour was monitored to measure attraction to or avoidance of the cues and water samples were collected to determine changes in the pattern of pulsatile urea excretion. Toadfish did not have a directional response to any of the chemical cues, but significantly more toadfish pulsed within 7 hours of PC-SW delivery (67%) compared to control seawater (38%). Pulse frequency was also 1.6 times greater in response to PC-SW than control seawater. In comparison, toadfish did not pulse in response to the prey or alarm cues. Although none of the cues attracted or repelled toadfish, the results suggest that toadfish of either sex pulse to communicate with conspecifics when exposed to chemosensory cues from the opposite sex. Further experiments will investigate the role of sex hormones in the control of pulsatile urea excretion, possible reproductive pheromones released in pulses, and the adaptive significance of chemical communication in toadfish.

A12.28 MIXTURES OF ZINC, COPPER AND CADMIUM CAUSE DIFFERENT RESPONSES IN CAENORHABDITIS ELEGANS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

SOFIE MOYSON (UNIVERSITY OF ANTWERP, BELGIUM), KRIS VISSENBERG (UNIVERSITY OF ANTWERP, BELGIUM), GEERT BAGGERMAN (UNIVERSITY OF ANTWERP, BELGIUM), RONNY BLUST (UNIVERSITY OF ANTWERP, BELGIUM), STEVEN HUSSON (UNIVERSITY OF ANTWERP, BELGIUM)

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An increase in metal accumulation is seen the last decades, which can lead to serious health hazards for diverse animals including humans, resulting in a persistent (eco)toxicological concern. In contrast to the increasing understanding of the toxic effects of single metals, much less is known about their effects upon interaction, which frequently occurs in the natural environment. Especially soils, sediments and surface waters can be contaminated with metal mixtures. Since soil nematodes live within the interstitial waters of soil particles, they are in direct contact with dissolved contaminants and are thus good models for these toxicity tests. The aim of this study was to gain insights into the sensitivity to the selected metals (Cu, Cd, Zn), and to investigate whether and how these sensitivities are affected in mixture exposure scenarios. To do so, we fully exploited the benefits of the free-living soil nematode *Caenorhabditis elegans* as a unique model to investigate the effects of metal exposure on population growth and locomotory behaviour. Crawling speed on agar plates and trashing behaviour in liquid medium was evaluated by using video tracking. These experiments were preceded by mortality experiments to determine LC20 concentrations used in the behavioural tests. *C. elegans* exhibited a different sensitivity to the three metals, both as individual metals as in combination. Different interaction effects were observed for the mixtures ZnCd, ZnCu, CuCd and ZnCuCd. Our study showed that even at low concentrations the locomotion, both on agar plates and in liquid medium, was disturbed.

A12.29 SPATIAL SUMMATION IN HAWKMOTH LAMINA MONOPOLAR CELLS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

ANNA STÖCKL (AALTO UNIVERSITY, FINLAND; LUND UNIVERSITY, SWEDEN), DAVID O'CARROLL (LUND UNIVERSITY, SWEDEN), ERIC WARRANT (LUND UNIVERSITY, SWEDEN)

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Many nocturnal animals rely on vision as their primary sense. The challenging conditions at night - low signal and a high noise background - are met by adaptations in the eyes and retina. In insects, neural processing in the brain further increases sensitivity by summing visual signals in space and time, thus boosting the correlated signal while reducing the uncorrelated noise, yet at the expense of spatial and temporal resolution. The neurons responsible for this summation remain unknown, although clues exist that lamina monopolar cells (LMCs) - found in the first visual processing area of the insect brain - have the necessary morphology to perform spatial summation. Here we give the first physiological evidence

to support this hypothesis. We recorded from LMCs intracellularly and characterised their spatial responses at a range of intensities in the hawkmoth *Macroglossum stellatarum*. The LMCs responded to 100 times dimmer light levels than the photoreceptors they receive information from, and their spatial resolution decreased with light intensity, strongly suggesting that they carry out spatial summation. Moreover, the spatial responses of the LMCs at different light intensities closely matched the extent of spatial summation previously measured in the motion vision system of this hawkmoth species. Finally, the spatial receptive fields of LMCs closely matched the lateral extents of their dendrites, suggesting that the lateral dendrites of LMC are responsible for integrating information in space. Our work not only answered a decade-old question in dim light vision, but also provides new insights into spatial information processing in insects.

A12.30 CHOLESTEROL-RICH MEMBRANE RAFTS REGULATE BASAL ATP RELEASE AND ALTER CONTRACTILITY OF THE BLADDER

WEDNESDAY 5 JULY, 2017 POSTER SESSION

DARRYL G KITNEY (UNIVERSITY OF BRISTOL, UNITED KINGDOM), CHRISTOPHER H FRY (UNIVERSITY OF BRISTOL, UNITED KINGDOM)

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Basal ATP is released from the mucosa of the bladder wall and is important in many physiological responses including the regulation of spontaneous contractions, and its mechanism of release is unknown. Cholesterol-rich microdomains may be responsible for regulating this ATP release. We hypothesise, 'depletion of cholesterol-rich microdomains inhibits the release of ATP and reduces contractility'. Pig bladders obtained from a local abattoir was dissected into strips and superfused with heated Tyrode's solution. Preparations were left to stabilise for 60 min and then superfused with methyl β -cyclodextrin (MCD) or a Tyrode's control for 90 min. Superfusate samples were taken 5 min before addition of MCD or control and every 30 min after. Superfusate ATP measurements were done using a luciferin-luciferase bioassay and read in a luminometer. Bladder contractions were measured using a force-transducer. Comparisons between data sets were done using a two-way ANOVA with Sidak's multiple comparison. The depletion of cholesterol-rich microdomains using MCD (15 mM) significantly inhibited basal ATP release between 30-90 min compared to pre-control and Tyrode's (vehicle) control. There was an opposite effect on basal tone and spontaneous contractions showing significant increases between 60-90 min. Cholesterol-rich microdomains seem to have an important role in regulating basal ATP release which is independent to the effects on contractility. It is important to further explore the release of ATP and the regulation of contractility by cholesterol-rich microdomains that control normal bladder physiology.

A12.31 THE GENERATION OF BLADDER WALL SPONTANEOUS CONTRACTIONS IS INFLUENCED BY DIFFUSIBLE SUBSTANCE(S) FROM THE MUCOSA

TUESDAY 4 JULY, 2017 POSTER SESSION

DARRYL G KITNEY (UNIVERSITY OF BRISTOL, UNITED KINGDOM), CHRISTOPHER H FRY (UNIVERSITY OF BRISTOL, UNITED KINGDOM)

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The bladder possesses spontaneous contractions (SC) which has been an integral part of bladder physiology, but its origin and generation is still unknown. It is known that the mucosa influences these SC as the detrusor smooth muscle (DSM) layer alone lacks activity. Whether this influence is by the release of a diffusible substance or cell-to-cell communication remains unclear. We hypothesise, 'bladder SC are enhanced by the diffusion of an excitatory chemical released from the mucosa'. *In vitro* contractility studies were done using porcine bladders obtained from a local abattoir. The bladder was dissected as an intact (mucosa+detrusor) preparations or the mucosa was removed (DSM). Bladder preparations were attached to a force transducer in a tissue bath and superfused with warmed Tyrode's. DSM preparations were allowed to equilibrate for 1 h prior to intervention. The mucosal layer (from the same preparation) was then laid on top of the DSM for 1 h, and removed again for a further 1 h. Experiments were done with the layers reversed, and time controls are intact bladders. DSM preparations did not produce any SC within 1 h. The addition of the mucosa initiated the generation of SC which persisted even if the mucosa was then removed. SC from the mucosa was not influenced by the DSM. Intact preparations had consistent SC which lasted to similar magnitude for 3 h. The mucosa of the bladder wall shows a fundamental influence in the initiation and generation of SC which is, in at least, part controlled by a diffusible substance.

A12.33 OXYGEN DICTATED THE EVOLUTION OF THE VERTEBRATE EYE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

CHRISTIAN DAMSGAARD (AARHUS UNIVERSITY, DENMARK), HENRIK LAURIDSEN (AARHUS UNIVERSITY, DENMARK), ANETTE M D FUNDER (AARHUS UNIVERSITY, DENMARK), JESPER S THOMSEN (AARHUS UNIVERSITY, DENMARK), THOMAS DESVIGNES (UNIVERSITY OF OREGON, UNITED STATES), DANE CROSSLEY (UNIVERSITY OF NORTH TEXAS, UNITED STATES), DO T T HUONG (CAN THO UNIVERSITY, VIETNAM), WILLIAM DETRICH (NORTHEASTERN, UNITED STATES), ANNEMARIE BRÜHL (AARHUS UNIVERSITY, DENMARK), JENS R NYENGAARD (AARHUS UNIVERSITY, DENMARK), MICHAEL BERENBRINK (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM), TOBIAS WANG (AARHUS UNIVERSITY, DENMARK), MARK BAYLEY (AARHUS UNIVERSITY, DENMARK)

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The high metabolic demands of the vertebrate eye require efficient oxygen supply, yet most non-mammalian retinæ are avascular. Hence, evolution of improved visual performance by virtue of thicker retinæ and larger eyes requires a parallel augmentation for retinal oxygenation. To overcome the large diffusive barrier for oxygen, three morphological and physiological mechanisms have allowed the evolution of larger eyes and retinal thickness: i) oxygen secretion by the combination of Root effect haemoglobins and a choroid rete, ii) intraretinal- or iii) vitreous capillarization. By reconstructing the evolution of retinal oxygen supply and retinal- and eye morphologies across an 87-species vertebrate phylogeny, we show that the three distinct pathways for retinal oxygenation evolved multiple times among vertebrates, and we show parallel evolution of the capacity for retinal oxygenation and eye/retinal morphology. Oxygen secretion seems to be the most efficient mechanism for retinal oxygenation allowing avascular fish retinæ thickness to vastly exceed that of tetrapods. We show that Root effect was lost seven times independently, and the conservation of retinal oxygenation after such losses, required compensations by the two alternative routes. This reflects the same phylogenetic constraint experienced by stem tetrapods, where evolution of larger eyes was achieved by either intraretinal- or vitreous capillarization. Our analysis illustrates the limit in solutions available for increasing visual performance, and that the phylogenetic context determines which physiological pathways that enable sufficient oxygen delivery to evolve eye- and retinal complexity, and hence provides novel insight into on the evolution of the vertebrate eye.

A12.34 A MULTI-TASKING STOMACH: DEFENCE THROUGH INFLATION AND DIGESTION THROUGH ACID-PEPSINOGEN PRODUCTION. THE CASE OF THE SARGASSUM FISH, *HISTRIO HISTRIO*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The stomach is characterized by the occurrence of acid (HCl) and pepsin-producing glands that enable acid-peptic digestion. As evolutionary pressures fluctuated over time, some organisms appear to have lost the gastric phenotype. Examples can be found in multiple lineages of vertebrates, such as mammals (e.g. monotremes) and numerous fishes as the chimaeras (Holocephali), the lungfishes (Dipnoi) and a great number of teleosts. This loss of function is intriguing, as the stomach's development is amongst the major innovations that arose within the early gnathostomes. In the Tetraodontiform puffer and porcupine fishes (Diodontidae and Tetraodontidae) stomach inflation for defence correlates with the loss of the gastric phenotype and acid-peptic genes and may be a potential driver for loss of the gastric phenotype. The sargassum fish *Histrio histrio* (Lophiiformes) displays a similar inflation response and this work aims to determine if inflation is a possible driver for gastric function loss in this species. The stomach phenotype was confirmed by histology and immunohistochemistry through the detection of gastric glands, zymogen granules, and the gastric proton pump H⁺/K⁺ ATPase immunoreactivity. In addition, through molecular methods, genes associated with the gastric function: *atp4a* (the α subunit of the H⁺/K⁺ proton pump), and two pepsinogens *pgc* (pepsinogen C) and *pga* (pepsinogen A) were detected in the stomach. *Atp4a* protein expression was confirmed through Western blotting. Taken together, these results indicate a fully functional stomach in *H. histrio*, demonstrating the possibility of coexistence of both acid-peptic digestive and inflation phenotypes

A12.36 PREFERRED TEMPERATURE IS SIZE DEPENDENT IN EUROPEAN PERCH *PERCA FLUVIATILIS*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Behavioural thermoregulation is utilised by fishes to seek out beneficial thermal habitats and correlates with the optimal temperature of the animal. Therefore, the trait is of great interest for a variety of scientific fields, including conservation biology, fisheries, and aquaculture. The temperature preference of European perch *Perca fluviatilis*, a eurythermal freshwater fish, was determined in a two tank choice system, where the temperature changed dynamically according to the position of the fish. The determined preferred temperature ranged between 22.0 and 28.6°C and was significantly decreasing with fish size (body mass ranging between 3 and 54 g) in a logarithmic fashion. As gill surface to body volume ratio also decreases with body size in many fishes, gill surface to body volume ratio may be limiting maximum oxygen uptake, and consequently be the limiting factor for the scope for activity as reflected in the temperature preference. These results show that fish size is a significant covariate in temperature preference of fishes, and could have a large influence on the spatial distribution in situ of a species throughout its life history.

A12.37 USING A MULTI-STRAIN PROBIOTIC TO MEASURE THE EFFECTS ON GROWTH, MORTALITY, FOOD CONVERSION RATIOS (FCRS), AND BEHAVIOR IN RED DRUM (*SCIAENOPS OCELLATUS*)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Bacteria are important to a functional digestive system, and the use of probiotics, defined as "good bacteria", has become more common in the last 10 years to help re-establish the gut flora after treatment of antibiotics. Little work, however, has been done using probiotics in commercial marine fish culture, e.g. in red drum. Red drum (*Sciaenops ocellatus*) a commercially important carnivorous marine fish has a short gut and rapid food transport. Rapid digestion and assimilation rates are important to rapid growth. The goals of the current study was to determine the effects of probiotics on growth & mortality rates, food conversion ratios (FCRs), behaviour, and to evaluate gut health. The individuals treated with probiotics (1.0x10⁸ CFU/g) until day 56 had an overall increased growth of 33.7% compared to controls, larger than probiotics till day 28, but not significantly different. FCR calculations show the control fish require almost double the amount of feed to make 1 g of tissue

mass compared to probiotics treated fish. Behaviour was compared on an aggression index created in the lab based on observations. Future work will include quantifying the effects of probiotics on the diversity of the red drum gut microbiome using deep sequencing studies. Together these investigations will provide an index of health of the red drum while being treated with probiotics. This will aid us in determining if probiotics is a viable and sustainable alternative to antibiotics as a growth and fish health enhancer.

A12.38 DEALING WITH DANGER: INVESTIGATING HOW PREY RESPOND TO CHANGES IN THREAT

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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For every animal, finding food and avoiding predation are essential to survival. This need to locate prey while avoiding predation influences decisions and shapes predator-prey behaviour and distribution. Despite the importance of these predator-prey interactions, many studies have overlooked the interplay between predator and prey behaviour and assessed behaviour of one in the absence of feedback from the other. By treating one side as non-interactive agents, previous studies have potentially missed the dynamic manner in which predator and prey adjust or adapt their behaviour. In this experiment, jade perch predators, *Scortum barcoo*, were placed into clear circular arenas surrounded by groups of 10 mosquitofish, *Gambusia holbrooki*. Both predator and prey movements were recorded for 12 minutes. We found that prey movement correlated to predator movement with the time lag in response to predator movement changing as a function of time. Furthermore, by analysing each prey within the group separately, we found that this relationship between predator and prey locomotion would switch when predators tracked prey, demonstrating flexibility in the relationship between predator-prey behaviour. These results demonstrate that predator and prey adjust their behaviour in response to each other, which can ultimately reshape the current perspective on predator-prey interactions.

A12.39 NADPH OXIDASE SUBTYPE EXPRESSION AND ROS GENERATION IN THE MOUSE URINARY BLADDER

WEDNESDAY 5 JULY, 2017 POSTER SESSION

MAX ROBERTS (UNIVERSITY OF SURREY, UNITED KINGDOM),
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Reactive oxygen species (ROS) produced *in vivo* can irreversibly destroy or alter the function of biological molecules, where this process is widely accepted as a major contributor to aging. NADPH oxidases (NOX) have gained increasing interest, as they are the only known enzymes to produce ROS as a primary function, for the first time indicating ROS to also have important physiological functions. Few studies have explored the role of ROS in the bladder. Since the elucidation of the sensory role of the urothelium—the inner mucosal lining of the bladder, there has been intense interest in identifying new physiological regulators in this structure. We investigated for the first time, the expression and functional importance of NOX enzymes in the bladder mucosa.

Tissues were obtained from young WT mice (male C57BL/6). Immunohistochemistry and western blotting were employed to detect NOX subtype expression, and lucigenin-enhanced chemiluminescence method used to evaluate superoxide production in the presence of various inhibitors. NOX1, 2 and 4 were expressed throughout the bladder, with highest expression observed in the urothelium. Bladder urothelium produced significantly more superoxide than other tissues assessed. Inhibitors revealed that mitochondria are responsible for approximately 30–35% of superoxide production, with NOX enzymes accounting for 70%.

These findings demonstrate high release of ROS from the urothelium not observed in other tissues, where NOX are responsible for a significant proportion, possibly implicating NOX-derived ROS as important physiological regulators of bladder function.

A12.40 THE GILL PARASITE *PARAMOEBA PERURANS* COMPROMISES AEROBIC SCOPE AND SWIMMING CAPACITY IN ATLANTIC SALMON *SALMO SALAR*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The parasite *Paramoeba perurans* is an etiological agent of amoebic gill disease (AGD), a serious problem in seawater salmonid aquaculture globally. Other finfish species are also infected and infection events are associated with periods of unusual high temperatures. Currently little is known about the impact of AGD on wild fish, but in a time with global warming and increasing aquaculture production a potential threat could be on the rise. A better understanding of the pathophysiology of infected fish is therefore warranted. In this study groups of Atlantic salmon without and with severe AGD were tested in a large swim tunnel respirometer

in seawater at 13°C to assess swimming capacity, oxygen uptake and blood parameters. The critical swimming speed was reduced from 3.0 BLs⁻¹ in controls to 2.5 BLs⁻¹ in infected fish. Standard metabolic rates were similar between groups, but the maximum rate of oxygen uptake was drastically reduced in AGD fish, which resulted in a smaller aerobic scope of 260 mg O₂ kg⁻¹ h⁻¹ compared to 410 mg O₂ kg⁻¹ h⁻¹ in healthy fish. Furthermore AGD fish had lower haematocrit and [haemoglobin], but similar condition factor to controls. Before swim trials AGD fish had higher plasma osmolality and elevated plasma [Na⁺] and [Cl⁻] compared to controls indicating reduced capacity to maintain ionic homeostasis, while cortisol levels were higher in AGD fish both before and after swim trials. These results show that AGD inhibits gill function, is a significant stress factor, and decreases swimming performance.

A12.41 INTERROGATION OF THE GULF TOADFISH INTESTINAL PROTEOME RESPONSE TO HYPERSALINITY EXPOSURE USING MASS SPECTROMETRY

WEDNESDAY 5 JULY, 2017 POSTER SESSION

MARTIN GROSELL (ROSENSTIEL SCHOOL OF MARINE AND ATMOSPHERIC SCIENCE UNIVERSITY OF MIAMI, UNITED STATES), KEVIN L SCHAUER (ROSENSTIEL SCHOOL OF MARINE AND ATMOSPHERIC SCIENCE UNIVERSITY OF MIAMI, UNITED STATES), AALEKHYA REDDAM (ROSENSTIEL SCHOOL OF MARINE AND ATMOSPHERIC SCIENCE UNIVERSITY OF MIAMI, UNITED STATES), ELVIS G XU (UNIVERSITY OF CALIFORNIA RIVERSIDE, UNITED STATES), LISA M WOLFE (COLORADO STATE UNIVERSITY, UNITED STATES)

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The marine teleost intestine is a multifunctional organ involved in numerous processes including digestion and osmoregulation. Several large-scale investigations of the marine fish intestine have been completed on the transcript level, but in-depth investigations of the proteome are lacking. Here, a shotgun proteomics methodology employing isobaric tandem mass tags (TMT) was used to interrogate the anterior intestine, posterior intestine, and intestinal fluid proteomes of Gulf toadfish (*Opsanus beta*) acclimated to normal (35 ppt) or hypersaline (60 ppt) seawater. Relative protein abundance between the intestinal segments and the intestinal fluid was also investigated using label free quantitative methods. Protein products from nearly 3,000 unique toadfish loci were identified and quantified between the tissues, including numerous involved in the regulation of ion transport, digestion, nutrient uptake, and CaCO₃ precipitation. Several proteins involved in digestion and nutrient absorption were found at lower abundance in the hypersalinity acclimated fish, suggesting that digestive processes in the intestine may be reduced to compensate for the increased energetic demands imposed by the osmotic stress. Additionally, several proteins found in the organic matrix of the intestinally derived CaCO₃ precipitates showed differential abundance between salinities, suggesting they may play a role in regulating precipitation *in vivo*, as CaCO₃ production is increased more than 2-fold during hypersalinity exposure. The results from this experiment provide new insights into the regulation of several osmoregulatory processes that warrant further investigation, and provide data that can be used by researchers interested in wide-ranging aspects of intestinal physiology.

A12.42 HIGH RESOLUTION 3D IMAGING OF A PARASITIC WASP OVIPOSITOR BASE SHOWS ITS ROLE IN PROBING

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Parasitic wasps use highly specialized ovipositors to drill in a variety of substrates to reach hidden hosts. These ovipositors consist of a base inside the abdomen and three long, slender elements forming a needle-like structure. These elements, or valves, are interconnected and can slide along each other. This sliding motion is important, as it has been hypothesised that alternating valve movement simplifies insertion and is used to steer the ovipositor. As the valves do not contain any muscles, all movement must be initiated at the base. Currently it is unclear how valve movement is controlled. We analyse the working mechanism of the ovipositor apparatus in the wasp *Diachasmimorpha longicaudata*, in four stages of probing, namely rest position, and probing position with a dorsal valve, ventral valve and equal valve protraction. We used synchrotron X-ray microtomography to create 3D reconstructions of the ovipositor apparatus. These showed that the valves and their interconnections extend inside the abdomen, forming a semicircular structure to which two pairs of plates, termed valvifers, rigidly attach. This base can rotate around a hinge to get it into probing position. An additional hinge is located between the valvifers which allows for, and also limits, the relative movement of the valves. The angle between the valvifers clearly changes with the movement of the valves. Several muscles attach to the valvifers, this suggests that these muscles induce valve movement. Our findings improve the understanding of the Hymenopteran probing kinematics and is a first step in understanding the valve control mechanism.

A12.44 COMPARISON OF MULTIPLE ACID COMPOUNDS IMPACTS ON THE ONTOGENESIS OF PURPLE SEA URCHIN (*ANTHOCIDARIS CRASSISPINA*)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The sea urchin larvae have shown their sensitive reaction to CO₂-induced reductions in seawater pH. Under the experiment condition, the levels of pCO₂ and [H⁺] were highly co-related, but they may have different physiological and impeding effects along sea urchin development. This study aimed to investigate whether the higher [H⁺], but normal pCO₂, can reduce the growth of sea urchin larvae or not. The purple sea urchin (*Anthocidaris crassispina*) gametes were fertilized and kept at 25°C under the normal or the acidified seawater

(pH 7.8) induced by CO₂, HCl, and H₂SO₄, where HCl treatment did not change seawater [Cl⁻] significantly. The pluteus larvae were collected from each group at 24, 48 and 72 hr after fertilization, and their length of the longest skeletal rod were measured. The results showed that both CO₂- and HCl-induced acidification significantly affect the larvae development. Moreover after incubation for 48 hr, the HCl treated larvae has significantly longer skeletal rod than the CO₂ counterparts. On the other hand, H₂SO₄ perturbation led to high mortalities before 48 hr, and the survivors grew the slowest among treatments. This comparative study inferred that higher pCO₂ might have synergistic role on the physiological impacts on high [H⁺] environment, especially for the early stages. Furthermore, the observations from H₂SO₄ acidified group indicated the industrial pollutants can severely affect the sea urchin larvae that we should pay more attention to.

A12.47 PTEROICIDINS: ANTIMICROBIAL PEPTIDES RELATED TO PISCIDINS IN THE VENOMOUS FISH *PTEROIS VOLITANS*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Piscidins are fish-specific antimicrobial peptides (AMPs) that play important roles in the innate immune defence system. They can be found in a wide range of fish species. Piscidins exhibit variability in their sequences, yet with a similar amphipathic α -helical conformation. We used a transcriptomic approach to analyse the venom apparatus of the lionfish (*Pterois volitans*), and identified three transcripts encoding peptides related to piscidins and called pteroidins-A, B, C. Pteroidin-A shares relatively high sequence identity (55%) with moronecin, the first piscidin identified from the hybrid striped bass. Mass spectrometry analysis confirmed the presence of pteroidin-A, which is 21 amino acid residues long. Pteroidin-B seems to be closer to piscidin-4 from hybrid striped bass, which is an unusually large member of the piscidin family with a length of 44 amino acid residues. The last pteroidin (C) shares low identity with the currently known piscidins, and seems to be more original. Mass spectrometry analysis did not allow us to determine the lengths of pteroidins B and C. Finally, we synthesized several forms of pteroidins with different lengths to evaluate their antibacterial and hemolytic activity. Each peptide exhibited a different activity spectrum, suggesting that peptide length and physicochemical characteristics played a determining role in the interactions with bacterial or erythrocyte membranes.

A12.48 DEMYSTIFYING ANIMAL 'PERSONALITY' (OR NOT): WHY INDIVIDUAL VARIATION MATTERS TO EXPERIMENTAL BIOLOGISTS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Animal 'personality', defined as repeatable inter-individual differences in behaviour, is a concept in biology that faces intense controversy. Critics argue that the field is riddled with terminological and methodological inconsistencies and lacks a sound theoretical framework. Nevertheless, experimental biologists are increasingly studying individual differences in physiology and relating these to differences in behaviour, which can lead to fascinating insights. We encourage this trend, and highlight some of the benefits of estimating variation in (and covariation among) phenotypic traits at the inter- and intra-individual levels. We focus on behaviour while drawing parallels with physiological and performance-related traits. First, we outline some of the confusion surrounding the terminology used to describe repeatable inter-individual differences in behaviour. Second, we argue that acknowledging individual behavioural differences can help researchers avoid sampling and experimental bias, increase explanatory power and, ultimately, understand how selection acts on physiological traits. Third, we summarize the latest methods to collect, analyse and present data on individual trait variation. We note that, while measuring the repeatability of phenotypic traits is informative in its own right, it is only the first step towards understanding how natural selection and genetic architecture shape intra-specific variation in complex, labile traits. Thus, understanding how and why behavioural traits evolve requires linking repeatable inter-individual behavioural differences with core aspects of physiology (e.g. neurophysiology, endocrinology, energy metabolism) and evolutionary biology (e.g. selection gradients, heritability).

A12.49 INTERSPECIFIC VARIATION AND PLASTICITY IN HEMOGLOBIN NITRITE REDUCTASE ACTIVITY AND ITS CORRELATION WITH OXYGEN AFFINITY IN VERTEBRATES

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Deoxygenated hemoglobin (Hb) is a nitrite reductase that reduces naturally occurring nitrite to nitric oxide (NO), supplying physiological relevant NO under hypoxic conditions. The nitrite

reductase activity is modulated by the allosteric equilibrium between the R and T structures of Hb that also determines oxygen affinity. We investigated nitrite reductase activity and O₂ affinity in Hbs from ten different vertebrate species under identical conditions to disclose interspecific variations and allow an extended test for a correlation between the rate constant for nitrite reduction and O₂ affinity. We also tested plastic changes in Hb properties via addition of T-structure-stabilizing organic phosphates (ATP and GTP). The decay in deoxyHb during its reaction with nitrite was exponential-like in ectotherms (Atlantic hagfish, carp, crucian carp, brown trout, rainbow trout, cane toad, Indian python and red-eared slider turtle), while it was sigmoid in mammals (harbor porpoise and rabbit). Typically, hypoxia-tolerant species showed a faster reaction than intolerant species. Addition of ATP and GTP decreased O₂ affinity and reduced the rate of nitrite reduction in a concentration-dependent manner. The initial second order rate constant of the deoxyHb-mediated nitrite reduction showed a strong curvilinear correlation with oxygen affinity among all ectothermic vertebrates, and the relationship also applied to plastic variations of Hb properties via organic phosphates. The relationship predicts high nitrite reductase activity in hypoxic tolerant species with high Hb-O₂ affinity and reveals that the decrease in erythrocyte ATP and/or GTP during acclimation to hypoxia in ectotherms increases the erythrocyte NO generating capacity [CBP-A 206 (2017) 47-53].

A12.50 NOVEL INSIGHTS INTO LOCUST DISCONTINUOUS GAS EXCHANGE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Insect discontinuous gas exchange (DGE) cycles comprise three phases, defined by spiracular state: close, flutter and open. However, spiracle status has rarely been monitored directly. Instead, it is often assumed based on recorded respiratory gas traces. In this study, we directly monitored muscular activity associated with DGE in locusts. We carried out electromyogram recordings from the closer muscle of the second thoracic spiracle and from abdominal ventilation muscles simultaneously with recording of CO₂ emission in a fully intact locust during DGE. Our findings suggest that the locust's DGE does not correspond well with the classical spiracle state model. Furthermore, we found that the DGE pattern correlates with and closely follow the ventilation motor pattern. During the open phase, when CO₂ emission rate is the highest, the spiracles do not remain open; instead, they open and close rapidly. This spiracle activity coincides with in-phase abdominal ventilation and shows alternation with the last abdominal spiracle, thus facilitating a unidirectional flow of air along the body. The flutter phase of locusts, rarely seen in normoxia, appeared as short CO₂ bursts prior to the open phase, again fully synchronized with ventilation. Hypoxia resulted in an increase in the number of these bursts, as well as the overall duration of the flutter. Ventilation bouts during the close phase are accompanied by increased spiracle closure activity. We offer a modified mechanistic model for DGE in actively ventilating insects such as the locust, incorporating ventilation and spiracles state.

A12.51 MOLECULAR STRESS RESPONSE IN *LUMBRICUS TERRESTRIS*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The issue of anthropogenic soil pollution, growing industries, and agriculture is very widespread and therefore receiving more attention in the recent years. One major soil pollutant is Cadmium (Cd), a highly toxic and carcinogenic heavy metal. Earthworms are important bioindicators and well suited to examine the cellular and molecular mechanisms, which enable them to survive in heavy metal contaminated soils. The species *Lumbricus terrestris* has been applied in the present study to characterize the fitness and biomarker response, as well as the expression of detoxification-related genes and epigenetic modifications in a long-term experiment upon environmentally relevant Cd exposure. Our results revealed that the fitness parameters and the biomarkers are mainly unaffected by Cd, maybe as a result of the activation of detoxification mechanisms like the expression of Metallothionein (MT). MTs are multifunctional, highly conserved proteins playing a major role in metal homeostasis and detoxification processes. A significant increase of MT gene expression was observed upon Cd exposure in a dose and time-dependent manner, whereas the expression of Phytochelatin Synthase (PCS) gene, another protein potentially involved in metal detoxification, was not affected at all. The MT expression level indicates a development of acclimation mechanisms. An increase of genome-wide DNA methylation has been found, which remained partly modified also after several months. In conclusion we could show that probably due to MT expression no immediate negative influence on individual earthworms could be observed, however, epigenetic modifications might be found in even moderately polluted environments.

A12.52 CHARACTERIZATION OF PEPTIDE TRANSPORTER SYSTEMS IN THE INTESTINE OF MOZAMBIQUE TILAPIA (*Oreochromis mossambicus*)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The peptide transporter (PepT) systems are well-known for their importance to protein absorption in all vertebrate species. These symporters use proton gradient at the apical membrane of the intestinal epithelial cells to mediate the absorption of small peptides. In fish, the intestine is a multifunctional organ, involved in osmoregulation, acid-base regulation, and nutrient absorption. We examined the effect of three environmental factors; salinity, pH and feeding, on the expression, activity and localization of three PepT transporters (PepT1a, PepT1b, PepT2) along the intestine of the Mozambique tilapia (*Oreochromis mossambicus*). Immunofluorescence analysis localized the all three transporters on the apical membrane of enterocytes, providing the first evidence for the participation of PepT2 in intestinal nutrient absorption. Both Quantitative real time PCR analysis and Immunofluorescence staining demonstrated that the two PepT1 variants are typical to the proximal intestinal section while PepT2 is typical to the distal intestinal sections. This first description of segment-specific expression and localization points to a complementary role of the different peptide transporters, corresponding to the changes in nutrient availability along the intestine. Gene expression and absorption activity assays showed that an increase in water salinity shifted the localization of the PepT genes transcription and activity down along the intestinal tract. Additionally, an unexpected pH effect was found on the absorption of small peptides, with increased activity at higher pH levels. This work emphasizes the relationships between different functions of the fish intestine and how they are affected by environmental conditions.

A12.53 EXPRESSION AND LOCALIZATION OF PEPTIDE TRANSPORTERS (PEPTS) DURING MOZAMBIQUE TILAPIA (*OREOCHROMIS MOSSAMBICUS*) LARVAL DEVELOPMENT

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Protein utilization is a major physiological process in fish, supplying amino acids for energetic requirements and tissue growth. While there are many studies on protein utilization in adult fish, less is known on utilization in larvae. During larval development, before exogenous feeding begins, the yolk sac is the only protein reservoir for these fundamental processes. Peptide transporter (PepT) is a trans-membranal protein that mediates intestinal absorption of di- and tri-peptide into the enterocytes, with a major role in protein utilization. In fish, there are two variants of the high capacity/low affinity isoform, PepT1a and PepT1b, and one variant of the low capacity/high affinity isoform, PepT2. We have shown that all three variants are expressed in adult Mozambique tilapia intestine and are localized in a physiological-complimentary manner. Aiming to investigate the role of these transporters in yolk-protein utilization during the larval development, we conducted a 19 day experiment in which we tracked the relative expression of the three PepT variants during the larval development. Custom made antibodies were used for visualization and localization of PepTs within the developing organs. All three transporters were expressed at 4-19 days post fertilization. The expression pattern of the three variants along this period of development imply complementary relationships, with a decrease in PepT2 expression in parallel to increase expression of the PepT1 variants. Immunolocalization of the PepTs in the developing larvae place them on the apical membrane of epithelial cells in the primordial intestine. The results will be presented within the context of embryonic development.

A12.54 EPIGENETIC INHERITANCE OF PAH RESISTANCE IN THE ZEBRAFISH (*DANIO RERIO*)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Uptake of polycyclic aromatic hydrocarbons (PAHs) occurs via gills or dietary intake in fishes, during oil exposure. In contrast to adaptive responses via genomic modifications, short-term epigenetic inheritance across a few generations may act as a bridge for survival while facing adverse environments. We used the zebrafish to test the proximate effects of PAH exposure in the P0 generation, and to then assess whether there was any transgenerational, epigenetic transfer of PAH-related effects to their F1 generation. After a 2-week acclimation period, adult zebrafish P0 were treated for 21 days with flake food spiked with water (control) or with low, medium or high

PAHs concentrations (~1500, 9000 or 17000 µg/g dw). The F1 larvae obtained from these parental treatments was subsequently exposed at 5 dpf to PAHs via water. No differences in adult body length, body and organ mass or condition factor were found. The F1 larvae from adults exposed to the higher levels of PAHs showed poor survival in clean water (up to 55% decrease) compared to larvae from control adults. In contrast, compared to F1 larvae from the control group, larvae from treated parents showed enhancement of PAH resistance (up to a 30% increase in survivorship) when challenged to PAH exposure via water. Epigenetic transgenerational inheritance of PAH resistance in the F1 larvae can arise from low levels of parental dietary PAH exposure. This adaptive response may help fish populations survive across a small number of generations while facing persistent environmental stressors.

A12.55 RNA-SEQ ANALYSIS OF SHELL REPAIR IN *MYTILUS EDULIS* IDENTIFIES CRITICAL ROLE OF TRANSPORTER PROTEINS IN BIOMINERALIZATION

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Bivalve shells are known to be secreted by the mantle tissue, but molecular processes of biomineralization in bivalve molluscs are poorly understood. In this study, we used high resolution RNA-Seq analysis of *Mytilus edulis* individuals during shell repair to gain an improved understanding of genes involved in biomineralization processes in the species. We drilled holes into the central shell area of the left valve and collected the edge and central mantle from the injured and non-injured valves of *Mytilus edulis* individuals for mRNA sequencing. Existing methods are limited to identifying and functionally characterizing shell matrix proteins from the organic matrix of shells. Rather than retroactively characterizing the expression levels of previously identified shell matrix proteins, our RNA-Seq approach (utilizing five biological replicates) made it possible to mine the mantle transcriptome and identify a large number of differentially expressed transcripts during shell repair. For the first time, we were able to identify transmembrane proteins as important players during shell formation: bicarbonate transporters, sodium neurotransmitter symporters and inward rectifying potassium channels were all highly expressed during shell repair in *Mytilus edulis*. Moreover, several transcripts with shell matrix domains were also found to be upregulated during shell repair, implicating the importance of organic components to shell formation. Finally, we were able to provide some clarification on haemocyte involvement in shell biomineralization, as we were able to distinguish between haemocyte and mantle specific domains during shell repair.

A12.56 MITOCHONDRIAL REGULATION PROTECTS THE ANOXIC TURTLE HEART FROM OXIDATIVE DAMAGE AFTER REOXYGENATION

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Reintroducing oxygen after a period of anoxia causes oxidative damage to tissues by production of reactive oxygen species (ROS). Freshwater turtle hearts (*Trachemys scripta*) are remarkably resilient to oxidative damage and suffers no damage from reoxygenation even after months of anoxia during winter hibernation. Preventing ROS production from mitochondria might be key to this resilience. In the mouse, inhibition of mitochondrial complex I by the post-translational modification S-nitrosation has been shown to inhibit ROS production upon reoxygenation. In this study, we tested the hypothesis that turtles prevent oxidative damage after anoxia/reoxygenation by limiting ROS production via S-nitrosation of complex I. We acclimated turtles to low temperature and anoxia or normoxia and analysed isolated heart mitochondria for respiration rate, ROS production, enzyme activity and S-nitrosation of mitochondrial proteins. We also used the mitochondria specific S-nitrosating agent MitoSNO to analyse the effect of S-nitrosation on turtle heart mitochondria *in vitro*. We found that anoxia acclimation does reduce ROS production and respiration rate of purified mitochondria, and that S-nitrosation of complex I inhibits activity and ROS production after anoxia/reoxygenation *in vitro*. However, activity and S-nitrosation of turtle complex I was not affected by anoxia acclimation *in vivo*. Instead, lower activity of the mitochondrial marker enzyme citrate synthase and lower maximal respiration rate of anoxic turtle mitochondria indicates down-regulation of the content of mitochondria in the turtle heart during anoxia. Reducing the content of mitochondria in the turtle heart during prolonged anoxia may prevent ROS production upon reoxygenation and protect against oxidative damage.

A12.57 DIVERSITY IS THE KEY - FUNCTIONAL ANALYSIS OF THE SEVEN MYOGLOBINS OF THE WEST AFRICAN LUNGFISH

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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From an evolutionary viewpoint, the lungfish is of high particular interest because it is considered the closest living relative of terrestrial vertebrates. Recent studies of the West African Lungfish *Protopterus annectens* revealed seven distinct myoglobin genes in this species. Such diversity appears to be unique throughout the animal kingdom. Myoglobin is a respiratory protein and one

of the best-studied proteins in biological science. It is well known for its function in oxygen (O_2) transport and storage in muscles. Furthermore, myoglobin helps reducing nitrite to nitric oxide (NO). Given its prominent role in O_2 supply and the unusual diversity of myoglobin genes in *P. annectens*, this species provides an ideal system to study the evolution of respiratory proteins in the transition from water to land. The myoglobin genes diverged early in lungfish evolution. Using quantitative qRT-PCR, we found that the myoglobin genes are differently expressed in various tissues, suggesting distinct functions. Also, the myoglobins revealed differences in their oxygen-binding kinetics, cooperativity, nitrite reductase rate and in autoxygenation. Untangling the roles of the different myoglobins in lungfish will further help understanding the functional plasticity of this protein in evolution.

A12.58 EFFECTS OF CORONARY LIGATION ON HEART RATE AND AEROBIC METABOLIC SCOPE IN RAINBOW TROUT, *ONCHORHYNCHUS MYKISS*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The capacity of the heart to pump oxygenated blood to the tissues is a fundamental determinant of how much aerobic metabolism can be elevated above resting levels (i.e. aerobic scope) during physically demanding challenges. Cardiac performance in turn is inherently dependent on the oxygen supply to the heart. In salmonids, a coronary circulation perfuse the outer compact myocardium with oxygenated arterial blood, while luminal venous blood provides oxygen to the inner spongy myocardium. However, the relative importance of these oxygen supply routes for cardiac and aerobic metabolic performance remains poorly understood. Here, we tested how chronic ligation of the coronary artery affected whole animal oxygen consumption (i.e. a proxy for metabolic rate) and heart rate before and after exhaustive exercise in rainbow trout implanted with heart rate data storage tags. Coronary ligation did not affect resting metabolic rate (45 ± 5 vs 49 ± 4 ml O_2 kg⁻¹ h⁻¹), but resting heart rate was elevated relative to sham operated trout (54 ± 8 vs 42 ± 11 beats min⁻¹), likely to compensate for a reduced cardiac contractility and stroke volume. However, while maximum heart rate during exercise was not affected (56 ± 6 vs 58 ± 6 beats min⁻¹), coronary ligation reduced the maximum metabolic rate (245 ± 27 vs 329 ± 13 ml kg⁻¹ h⁻¹). Thus, both aerobic scope and heart rate scope were reduced in ligated trout. These findings suggest that the presence of coronaries in rainbow trout allow an increase in aerobic metabolic scope, and is crucial for maintaining cardiac contractility and tissue oxygen transport during both resting conditions and physically demanding challenges.

A12.59 PARASITES AND A HOST'S SENSE OF SMELL: EFFECTS OF A NEW SPECIES OF TREMATODE *DACTYLOGYRUS OLFACTORIUS* (MONOGENEA, DACTYLOGYRIDAE) ON OLFACTORY PERFORMANCE OF FATHEAD MINNOWS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

EBRAHIM LARI (UNIVERSITY OF LETHBRIDGE, CANADA), RACHAEL WILBOURN (UNIVERSITY OF LETHBRIDGE, CANADA), CONE CONE (SAINT MARY'S UNIVERSITY, CANADA), CAMERON P GOATER (UNIVERSITY OF LETHBRIDGE, CANADA), GREG G PYLE (UNIVERSITY OF LETHBRIDGE, CANADA)

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Dactylogyrid flukes are common ectoparasites of fish and other vertebrate hosts. These flukes have direct life-cycles involving free-living larvae, making them amenable to experimental exposures in the laboratory. In the present study, *Dactylogyrus olfactorius* n. sp. (Monogenea) is described from the olfactory chamber of fathead minnows (*Pimephales promelas*) in Alberta, Canada. The pathophysiology of *D. olfactorius* inhabiting the olfactory epithelium of fathead minnow was examined through electro-olfactography (EOG) and olfactory sensory neuron (OSN) responses to chemical cues (L-alanine and taurocholic acid), in addition to histology and scanning electron microscopy (SEM) imagery of the olfactory epithelium. The EOG results showed that *D. olfactorius* inhibited the olfactory acuity of individual minnows. However, disinfected fish recovered their olfactory acuity within one week. SEM imaging showed that the density of cilia covering the olfactory epithelium was reduced in infected fish. Parasite-induced alteration to the olfactory epithelium is likely the underlying mechanisms leading to the observed reduction in fish olfaction. Histological analysis of the olfactory system showed that the number of mucous cells lining the olfactory epithelium dramatically increased in infected fish. Our results show, for the first time, that infection with a common parasite can influence the sense of smell of its host, potentially leading to declines in an individual's ability to detect predators, competitors, food, and mates.

A12.60 THE INNATE NATURAL GEN(Ε)IUS OF THE ACYTOTA: AN OBVERSE C-VALUE PARADOX

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Even considering that a lot of non-coding DNA is now known to be involved in sophisticated regulatory networks that are not yet understood, most living organisms maintain an excess of "junk" DNA. This lack of correlation between genome size and organismal complexity (the "C-value paradox") has intrigued biologists for decades. The genomes of the Acytota (the domain of cell-free replicons, which includes viruses, plasmids, bacteriophages and viroids) represent the opposite extreme. Acytota comprise an overwhelming majority of the biosphere's genetic resource and become "alive" only when provided with a suitable cellular environment, but they exert a vastly greater influence on the biosphere than would be expected from their minimal coding capacity. Unsurprisingly, those that have attracted most attention are potentially pathogenic. Rice Yellow Mottle Virus Satellite (RYMVS), a tiny (220 nucleotide) single stranded RNA molecule is the smallest of these.

We examined the behaviour of RYMVS in detail from a control systems perspective, with the aim of devising the simplest conceivable model of a biological nano-computer. Computational methods and resources were used to predict RYMVS genomes' response to change. The results showed that a population of RYMVS can behave like a perceptron, modifying its activities to maximise its survival. Physicochemical parameters that define the microenvironment influence "decisions" to replicate, transcribe, translate, or disassemble. Although viroids and virusoids are regarded as exclusively botanical phenomena, these observations also are very relevant to animals: neurons, for example, harbour multitudes of small circular RNA "perceptrons" that are presumed to process and store memory.

A12.61 CHEMICAL COMMUNICATION DURING SPAWNING IN THE SEA CUCUMBER *HOLOTHURIA ARGUINENSIS*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Synchronization of gamete release is vital for broadcast spawners; this is often achieved using pheromones. In echinoderms, however, little is known about the origin, identity or mechanisms of action of the chemicals involved. The current study addressed these issues during aggregation and spawning of the sea cucumber *Holothuria*

arguinensis. In a Y-maze assay, water conditioned by males attracted both males and females, whereas female-conditioned water did not. Coelomic fluid and gonadal extracts failed to replicate this effect. In a spawning assay, males spawning water (sea water with freshly released semen) induced spawning in both males and females, whereas females spawning water (sea water with freshly released ova) did not. Removal of sperm, by filtration, from males spawning water did not reduce its effect, suggesting that chemicals released with the sperm - putative pheromones - are responsible, rather than the sperm themselves. Initial attempts to isolate the active components by solid-phase extraction suggested that the spawning pheromone is multi-component; both hydrophilic and hydrophobic components are involved. However, at least one of the components is labile; biological activity was much reduced after four hours. Nevertheless, candidate molecules were detected by LC-MS in male, but not female, spawning water. Although further work is required to confirm chemical identity and biological activity of the compounds involved, the current study shows that male-released pheromones are important in aggregation and spawning of *H. arguinensis*.

A12.62 GLOBIN EXPRESSION PROFILE IN DIFFERENT TISSUES OF ZEBRAFISH EXPOSED TO HYPOXIA

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Globins are small respiratory proteins that bind oxygen and are involved in the oxidative metabolism. While the functions of myoglobin and hemoglobin in O₂ supply is well established, the physiological roles of the more recently discovered globins, i.e. neuroglobin, cytoglobin, androglobin, globin E, globin X, and globin Y, are still not fully established. Here, we have investigated the expression pattern and the response of the globins of the zebrafish (*Danio rerio*) to low O₂ levels in different tissues via quantitative RT-PCR. Neuroglobin, globin X and the duplicated cytoglobin genes (cytoglobin 1, cytoglobin 2), were differentially expressed in liver, heart, brain and gills. Globin X showed low levels in all tissues, while neuroglobin and cytoglobin 2 were most prominent in the brain, and cytoglobin 1 had its highest level in the liver. The globins showed a differential response to hypoxia, indicating different functions of neuroglobin, globin X, cytoglobin 1 and cytoglobin 2. Further, we study the functions of globins by generating knockout-mutants by means of CRISPR-Cas9.

A12.63 HYPOXIA TOLERANCE IN WHALES: A TRANSCRIPTOME ANALYSIS OF THE DIVING BRAIN

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Diving mammals are repeatedly exposed to low oxygen conditions (hypoxia), which may have immediate effects on the oxidative metabolism and thus on brain function. In most terrestrial mammals, even brief periods of reduced oxygen supply cause severe brain damage. However, many whales and seals must have developed strategies of hypoxia tolerance that allow them to dive for up to 2h without surfacing.

Physiological adaptations in diving mammals have been extensively examined, while little is known about the molecular mechanisms underlying the hypoxia tolerance of the diving brain. Recent studies have suggested a unique shift in the oxidative energy metabolism from neurons to astrocytes in deep-diving phocid seals which would explain an enhanced hypoxia tolerance. Such shift has not been observed for Cetaceans.

For a better understanding of the molecular adaptations in the cetacean brain, we compared Illumina-generated transcriptomes of the deep-diving pilot whale (*Globicephala melas*) and the killer whale (*Orcinus orca*) with a terrestrial relative, the cattle (*Bos taurus*). We analyzed the abundances of transcripts in the visual cortex and the cerebellum as well as mRNA expression levels of enzymes of the energy metabolism and typical stress-related genes. The results were compared with a similar approach, in which the brain transcriptomes of the hooded seal (*Cystophora cristata*) and the ferret (*Mustela putorius furo*) were compared. The results suggest divergent evolutionary strategies in whales and seals to sustain the demands for metabolic energy during cerebral hypoxia.

A12.64 JOINT CUSTODY AND ITS EFFECTS ON JUVENILE DEVELOPMENT IN MONGOLIAN GERBIL FAMILIES

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Mongolian gerbils (*Meriones unguiculatus*) are domesticated rodents previously used as laboratory animals but meanwhile receiving less attention despite their outstanding sociable behaviour. Gerbils establish pair bonds at ca. 6 weeks of age and then remain in these groups throughout their reproductive time span. They get into oestrus post partum so the female is concurrently pregnant and lactating. Interestingly, and contrary to many other rodents, the female not only tolerates the male inside the nest but rather, gerbil pairs seem to share the demands of raising young. What effects does pair wise raising of young have on maternal energy budgets and on milk production? What role does the male get in diminishing the adverse heat load produced by the females during lactation? We addressed this question by observing a total of 11 gerbil pairs from the age of 6 weeks to ca. 7-8 months while quantifying body weights, food intake, subcutaneous body temperatures as well as litter size and juvenile growth in each family. Our first data point towards a very successful reproductive rate with an average litter size at weaning amounting to 4.5 ± 1.9 pups. Lactation performance was close to 5 fold RMR compared to 6-8 fold in laboratory mice and 8-10 fold RMR in golden hamsters. We conclude that social behaviour largely affects maternal energy turnover and that female gerbils may have an advantage of the male presence in the nest.

A12.65 LOSING HOMEOSTASIS AT LOW TEMPERATURE: LOW TEMPERATURE IMPAIRS OSMOREGULATORY FUNCTION IN THE HINDGUT OF THE CHILL-SUSCEPTIBLE LOCUST, *LOCUSTA MIGRATORIA*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Loss of ion and water balance at low temperature has been suggested as a main cause of chill injury and mortality in insects. The hindgut of terrestrial insects is critically important for maintaining osmotic homeostasis as it selectively regulates solute- and water-balance. Using a chill susceptible insect, we here test the hypothesis that hypothermia causes disruption of the hindgut osmoregulatory capacity in a manner that is proportional to the homeostatic disruption observed under such condition. Using an *in vitro* everted gut sac preparation, we measured rates of ion (Na^+ , K^+ and Cl^-) and water flux across the hindgut under a range of thermal conditions allowing Q10 analysis. These findings were correlated to recordings

of the critical temperature at which ion and water balance is disrupted *in vivo*. Preliminary results show that water absorption is critically lowered during exposure to 0°C (a temperature that causes gradual loss of ion balance) and that the ability to reabsorb Na^+ was particularly impaired during hypothermia. Loss of osmoregulatory capacity seems to be primarily caused by reduced active transport as similar impairments are seen during exposure to anoxic medium. Furthermore, during the recovery period, ion and water flux rates returned to control values indicating the involvement of an active component to re-establishion- and water-balance. In conclusion, the capacity to maintain transport mechanisms in the hindgut at low temperature appears essential for insect cold tolerance. We therefore speculate that these mechanisms greatly influence adaptive and acclimation responses that alter insect thermal tolerance.

A12.66 THE EFFECT OF NUTRIENT LEVELS ON GROWTH AND TELOMERE DYNAMICS IN WILD ATLANTIC SALMON

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Fish that return from the ocean to spawn in fresh water are an important source of marine-derived nutrients for inland freshwater ecosystems. However, population declines, in combination with barriers to upstream migration, are reducing their nutrient input to rivers and lakes in many temperate and boreal regions. Using a wild system involving experimental manipulations of nutrient levels across replicate Scottish streams, we show that this 'oligotrophication' reduces the food supply, somatic growth and body condition of wild juvenile Atlantic salmon *Salmo salar*. Life-history theory predicts that greater investment in somatic growth may come at a cost to somatic maintenance. Moreover, a growing body of research suggests that measuring the length of telomeres, the protective caps at the end of eukaryotic chromosomes, may be a good way of quantifying somatic state, since a relatively short telomere length is indicative of poor biological state. We found that faster-growing fish had shorter telomeres, which supports the hypothesis that investment in growth can have negative consequences for somatic state. In addition, we also found significant differences in telomere length among streams, suggesting that the balance between somatic growth and maintenance may in turn depend on nutrient levels.

A12.67 DECIPHERING THE FUNCTION OF THE PLEUROPODIA IN INSECT EMBRYOS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Pleuropodia are peculiar appendages that transiently appear on the first abdominal segment of many insect embryos. Perhaps because they degenerate before the larva hatches and are completely missing in the genetic model insect *Drosophila* the pleuropodia escaped attention and their function is unclear. Experiments carried out more than a half century ago have shown that the pleuropodia secrete an enzyme degrading the serosal cuticle to enable hatching and synthesize a moulting hormone ecdysone. Ultrastructural studies by electron microscopy revealed that the pleuropodia contain a highly specialized epithelium that resembles epithelia involved in transport of water and ions (osmoregulation) and excretion. To decipher by modern techniques what functions the pleuropodia really have we isolated transcriptomes from several developmental stages of these appendages from the locust *Schistocerca gregaria*. Our RNAseq experiments support the function in osmoregulation and secretion of cuticle degrading enzymes, but not ecdysone synthesis. Our results newly reveal a role in immunity. These data will form a foundation for the subsequent physiological experiments.

A12.68 INVERTEBRATE IMMUNITY: ARENICOLA MARINA (ANNELIDA, POLYCHAETA) AS AN EXPERIMENTAL OBJECT

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Organization and functioning of immune system remains one of the least studied problems in invertebrate physiology. Comparative investigation of immune reacting ways in different phylogenetic groups of invertebrate animals is important for understanding of the immunity evolutionary patterns. Annelids (or "polychaetes") compose significant part of marine bottom ecosystems. They are involved in formation of food chains and also maintain the life cycles of several parasites. Annelids are characterized by metameric body structure, presence of coelomic cavity and a well-developed circulatory system. Combination of these features provides high level of adaptive plasticity and enables development of an effective immune (defensive) system. Free cell elements of the coelomic fluid, coelomocytes, constitute the main component of immune system in annelids. Coelomocytes offer recognition of foreign material and its subsequent elimination or isolation. Moreover these cells release humoral factors which are involved in immune response providing. *Arenicola marina*, the marine lug worm, is a common annelid species of cold-watered seas tidal zone. These worms are relatively large (length up to 15 cm) and their coelomic fluid contains great numbers

of coelomocytes. These features make *A. marina* a convenient object for the experimental work which is. We studied morphology of *A. marina* coelomocytes, their ability to perform phagocytosis and encapsulation of different foreign bodies using light, transmission, scanning and confocal microscopy. We also performed primarily investigations on the changes in ubiquitin-proteasomal system of these cells during the experimental inflammation. Our results create the basis for further experiments on this species.

A12.69 CARDIAC TISSUE OF AMPHIBIAN AND REPTILIAN CAVAL VEINS DEMONSTRATES ELECTRICAL EXCITABILITY AND AUTOMATICITY AND MAY CONTRIBUTE TO PACEMAKING

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The heart of amphibians and reptiles is characterized by myocardialized sinus venosus (SV). Similarly with SV, the wall of anterior caval veins (CVs) in various lower vertebrates may contain cardiac tissue. This tissue derives from the same embryonic precursors as myocardium of SV chamber. Bioelectrical properties of CV myocardium in lower vertebrates have not been investigated yet.

Optical mapping technique was used to evaluate action potentials (APs) waveform and excitation pattern in CVs of frog (*Rana temporaria*) and lizard (*Eublepharis macularius*). Optical signals were recorded using CCD camera (WuTech Inst.) from isolated multicellular preparations consisting of SV and anterior CV, perfused with adapted physiological solution (22°C), stained with di-4-ANEPPS (5 μM) and treated with blebbistatin (1 μM) to minimize the motion artefact.

Anterior CVs were electrically excitable in both species. APs were spontaneously initiated in SV in most cases, however excitation derived from CV or CV-SV junction zone was also observed in several experiments (3 of 7 in frogs, 2 of 6 in lizards). CV-derived rhythm was 31.6 ± 4.5 min⁻¹ in frog and 43.5–45 min⁻¹ in lizard. APs in the anterior CV are characterized by diastolic depolarization, low plateau duration and upstroke velocity, slow excitation conduction (0.02 ± 0.01 and 0.03 ± 0.01 m/s, respectively) either in frog or lizard.

In conclusion, CV myocardium at least in two different lower vertebrates (terrestrial and, predominantly, aquatic) is characterized by similar ability to generate pacemaker-like APs and probably may act as a cardiac pacemaker in particular conditions. This study was supported by RFBR [17-04-01921].

A12.70 ECOLOGICALLY-RELATED VARIATION IN GROWTH RATE AND MUSCLE PROTEIN DEGRADATION IN ATLANTIC SALMON OF NURSERY BROOKS AND NATAL RIVERS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Teleost fishes, including Atlantic salmon (*Salmo salar*), are indeterminate growers with maximal growth rate observed at the early years. Young salmonids of northern, low-production watercourses of the White Sea basin (North-Western Russia) are characterized by extremely low growth rate at the river period of their live comparing to their conspecifics from the lower-latitude watercourses. Salmon juveniles of 0+ and 1+ year-classes are differentiated in morphology and metabolism onto two ecological groups preferentially settling small brooks or natal rivers. Nursery brooks possess more favorable growth-promoting factors such as drifting food, water temperatures, etc. resulting in increased fish growth rates. Fish growth mostly relies on muscle protein synthesis and accumulation counterbalanced with protein degradation. Calpain and lysosomal proteolytic systems dominate in fish muscles while proteasomal digestion plays a minor role. Positive age-related correlation of calpain and cathepsin B activities in fish muscles and body growth increments was shown in salmon juveniles both of nursery brooks and natal rivers. Since growth rate was detected to be higher in juveniles in habitat brooks, the significant increase in proteolytic activities was expected; however, this pattern appears to expand only on salmon of 0+ year-class of but not later stages (1+). The research was supported by the Russian Scientific Foundation, project no. 14-24-00102.

A12.71 KNOCKDOWN OF MULTICOPPER OXIDASE 4 ELIMINATES THE PERITROPHIC MATRIX AND ALTERS LARVAL GROWTH AND ADULT MICROBIOME IN *DROSOPHILA*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Extracellular epithelial barriers allow organisms to regulate their interactions with the environment; one dynamic site of such interactions is the gut. Like many insects, *Drosophila melanogaster* produces an extracellular structure called the peritrophic matrix (PM), which lines the midgut and is theorized to aid in digestion and organismal defense. However, an absence of fly lines that lack a PM limits investigation of its physiological role. An RNAi screen of genes encoding putative cross-linking enzymes now reveals that silencing *multicopper oxidase 4 (mco4)* in the cardia, the site of PM synthesis, produces flies that lack a PM. *mco4* encodes a laccase and is expressed at high levels and nearly exclusively in the cardia. Experiments with *mco4* knockdowns revealed that lacking a PM significantly reduces adult body mass, extends the length of the third larval instar, and decreases the rate of larval weight gain. Consistent with a starvation phenotype, the nutrient-sensitive decrease in *Dilp8* expression is delayed in knockdown larvae. In adults, knockdown of *mco4* reduced microbiome abundance by 850-fold, as measured by 16S qPCR, and augmented the loss of gut bacteria caused by daily transfer of flies to fresh media. At the same time, expression of the antimicrobial peptide *diptricin* was increased 10-20 fold in knockdown flies, indicating hyperactivation of the immune system. These results suggest a role for the PM in larval nutrition and growth and in the maintenance of the adult gut microbiome. Supported by NSF grant IOS-1355087.

A12.72 3D ULTRASTRUCTURE STUDY OF Ca RELEASE PATHWAYS IN AVIAN CARDIOMYOCYTES

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Bird ventricular myocytes are long, thin and lack t-tubules. However, bird hearts achieve higher contractile rates and are capable of stronger pressure development than many mammalian and all ectothermic species. Excitation-contraction coupling in vertebrate hearts is underpinned by calcium (Ca^{2+}) diffusion between the Ca^{2+} release units, formed by clusters of ryanodine receptors present on the surface of the sarcoplasmic reticulum. In birds, Ca^{2+} release at peripheral couplings (PCs) in the sarcolemma must diffuse to internal corbular SR (cSR) structures for propagation of the Ca^{2+} signal. The distance Ca^{2+} must diffuse is therefore crucial in determining whether further Ca^{2+} -induced Ca^{2+} -release occurs deep inside these cells despite the absence of t-tubules. Electron tomography, a 3-dimensional (3D) microscopy technique was used to characterise the surface sarcolemma, including caveolae, and the SR network, in all four chambers of the heart of White Leghorn chickens. Nearest edge-edge distances between PCs and cSR, and geometric data about individual cSR were obtained and proportion of PCs in caveolae was assessed. Reconstruction demonstrates the pathways for Ca^{2+} diffusion throughout the cell. Large differences were found in the distances between cSR compared to those reported previously, possibly due to the methodology of data collection. Computer modelling was used to assess the relationship between distances among calcium release units, and calcium wave conduction velocity, as well as the stability of the propagation. This 3D structural characterisation will be valuable when functional work is done to study the underlying Ca^{2+} transient in the cardiomyocytes of this animal.

A12.73 ADRENERGIC CONTROL OF BLOOD PRESSURE AND VASCULAR RESISTANCE IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) ACCLIMATING TO SEAWATER

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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When salmonids migrate into seawater, they drink and absorb water in the intestine to maintain osmotic homeostasis. Gastrointestinal blood flow (GBF) has previously been observed to increase at least two-fold during this process, but it is unknown whether this is due to an increased dorsal aortic blood pressure (P_{DA}) or alterations in systemic vascular resistance (R_{SYS}), and what the underlying neuro-humoral causes of these hemodynamic changes are. Here, we measured PDA and cardiac output (CO) and calculated R_{SYS} in chronically seawater- and freshwater-acclimated rainbow trout (*Oncorhynchus mykiss*) *in vivo*. We also determined the adrenergic control of R_{SYS} at the different salinities by injecting the α -adrenergic agonist phenylephrine and the antagonist prazosin. Seawater-acclimated trout exhibited a significant reduction in R_{SYS} and P_{DA} compared to freshwater-acclimated trout, along with a tendency for an increased CO revealing that the elevated GBF following seawater acclimation is a consequence of reductions in gastrointestinal vascular resistance. Phenylephrine increased R_{SYS} and P_{DA} dose-dependently in both groups, and although R_{SYS} and P_{DA} were consistently lower in seawater-acclimated trout, the increases from baseline with phenylephrine were similar or larger, most likely indicating unaltered α -adrenoceptor sensitivity. Moreover, while prazosin reduced R_{SYS} and P_{DA} in both groups, both variables were still lower in seawater-acclimated trout after α -adrenergic blockade. Collectively, these findings suggest that reduced α -adrenergic tone alone was not responsible for the reduced R_{SYS} in untreated seawater-acclimated trout and that other local or hormonal vasodilatory factors reduce the vascular resistance of the gastrointestinal tract during seawater acclimation.

A12.75 ROLE OF THE CLOACA IN SALT AND WATER BALANCE IN ESTUARINE CROCODILES

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Crocodiles live across a range of salinities from freshwater to seawater. Seawater represents a dehydrating environment and crocodiles have to cope with an increased salt load. The kidney is not able to produce concentrated urine, and instead the cloaca with the lingual salt glands are responsible for excreting excess salt. While the salt glands are relatively well-studied, the mechanisms underpinning the regulation of water and electrolytes from the cloacal-colonic complex are largely unknown, thus the aim of this study was to characterize epithelial transport properties of the cloaca of estuarine crocodiles (*Crocodylus porosus*) in either fresh or saline water. Hatching crocodiles were acclimated in freshwater and 70% seawater for 6 weeks, and *in-vitro* activity, distribution, and expression of epithelial sodium-potassium ATPase (NKA), and vacuolar proton pump (V-ATPase) of the urodaeum and rectum were examined. Whole animal salt balance and hydration state via blood and muscle tissue analysis were also assessed to determine hydration and salt balance. We found a significant increase in transepithelial potential and active epithelial transport as indicated by increased short circuit current of the urodaeum under seawater conditions, however no difference within the rectum. The short circuit current of the urodaeum and the rectum appears to be mediated by sodium-hydrogen exchanger (NHE2) and is amiloride-sensitive. Muscle hydration, and blood plasma electrolyte and osmolality levels did not differ between treatments. Our results reveal the physiological roles played by the proximal intestine and cloacal segments in osmoregulation of estuarine crocodiles under seawater conditions.

A12.76 THYROID HISTOLOGY OF THE PACIFIC TREE FROG, *PSEUDACRIS REGILLA*

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Several amphibian populations have been declining in recent years and many species are on the brink of extinction. There is mounting evidence that anthropogenically derived environmental pollutants are contributing to amphibian population declines. Amphibians have long been used to study the effects of pollutants, and have unique attributes, such as increased skin permeability and thyroid hormone dependent metamorphic development, that warrant careful consideration during toxicity testing. The Organization for Economic Co-operation and Development (OECD) published a guideline in 2009 for a laboratory-based *in vivo* assay, the Amphibian Metamorphosis Assay. This assay is mainly used to screen for chemicals that disrupt the hypothalamic endocrine axis using methods specific to *Xenopus laevis*, a solely aquatic species found in Sub-Saharan Africa. Previous studies in our lab have examined the molecular and morphometric adverse endpoints in the AMA using a semi-aquatic amphibian species native to North America (*Pseudacris regilla*), but to date no histopathology has been reported in this species. Therefore, the objective of this study was to develop an atlas of thyroid development for Gosner stages 25 to 43 *Pseudacris regilla*. This atlas will serve as a reference for the normal histology of thyroid development throughout key periods during metamorphosis in this species, and further facilitate the use of this native North American amphibian in environmental toxicity testing regimes.

A12.77 SCIENTIFIC MISCONDUCT - ADVICE ON BEING A WHISTLEBLOWER

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Scientific discovery relies on high-quality research that is transparent, reproducible, and available for outside examination. Unfortunately, the current reward system can often select for poor quality research by incentivising practices that favour sensationalism, shortcuts, and novelty rather than quality and rigour. Globally, the annual science budget wasted on irreproducible research is estimated in the tens of billions of dollars. Such alarming levels of irreproducibility are not solely due to issues of poor statistical practices, confirmation bias, and selective reporting

- various forms of misconduct play large roles. Regardless of the reasons for which misconduct occurs, scientists have a moral and ethical obligation to report it when observed. Here, we present guidance for researchers who suspect or discover scientific misconduct, ranging from the initial stages of fact checking to the later stages of formal investigations. We use our own experiences as whistleblowers and lessons learned from conversations with other scientists who have had the unfortunate experience of witnessing fraud. We affirm that whistleblowing is one example of the self-correcting nature of science.

A12.78 TRAPDOORS APPARENTLY MODULATE THE PHYSICAL ENVIRONMENT OF SPIDER BURROWS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Many animal-built structures manage physiological fluxes between the occupant and the physical environment. Burrows are such structures, often assumed to protect the occupant from predators and to attenuate changes in temperature and relative humidity (RH) in the burrow cavity. We investigated whether trapdoors of desert-dwelling wolf spider burrows (*Lycosa sp.*) serve to maintain favourable conditions by removing trapdoors at different times. We tested the prediction that trapdoor-replacement is more frequent and done faster under adverse ambient conditions, when ambient air temperature (T_a) increases and RH decreases. In a preliminary comparison between seasons, we found that in summer, 88% of spiders ($n=9$) responded to early-morning trapdoor removal by closure with silk, whereas only 34% responded in winter ($n=41$). However, in summer, when trapdoors were removed at midday, only 22% of spiders responded ($n=9$). Thus, there is an apparent tradeoff between the cost of burrow repair, when daytime T_a is high and RH is low, and energy saved by closing the entrance. In addition, we investigated burrow ventilation using fluctuations in burrow cavity temperatures (measured with a descending array of tiny thermocouples) as a proxy for turbulence. We found stronger turbulence near the soil surface (<5 cm) in open than in covered burrows. We determined that eddies reached a depth of ~ 1 cm by cross-correlating temperature fluctuations in the burrow. The association between T_a and the spiders' response to trapdoor removal suggests that trapdoors indeed protect the burrow microclimate from the vagaries of surface conditions.

A12.79 BAROREFLEX FUNCTION IN FISH WITH UNIMODAL AND BIMODAL RESPIRATION

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The baroreflex is an important short-term regulator of cardiovascular homeostasis in vertebrates, where disturbances of arterial blood pressure promptly elicit an inversely proportional change in heart rate and systemic vascular resistance to restore arterial blood pressure. Considering that gravitational stress can impair animals' venous return and consequently arterial blood pressure in terrestrial, but not in aquatic environments, the present study's intent was to investigate whether fish species that were exposed to the influences of gravity during their evolutionary history are endowed with greater baroreflex sensitivity and efficiency compared to those that were not. We therefore studied the cardiac limb of the barostatic response in fish that perform (*Hoplerythrinus unitaeniatus* and *Anguilla anguilla*) and do not perform (*Hoplias malabaricus*, *Oreochromis niloticus* and *Pangasianodon hypophthalmus*) terrestrial locomotion. All fish were instrumented with a ventral aortic cannula to measure arterial blood pressure and heart rate, and after recordings from undisturbed animals, serial infusions of phenylephrine (10 to $80 \mu\text{g}\cdot\text{kg}^{-1}$) and sodium nitroprusside (20 to $160 \mu\text{g}\cdot\text{kg}^{-1}$) were administered through the catheter to correlate the change in arterial blood pressure with heart rate. The baroreflex functions were analysed through Kent's sigmoidal logistic function to identify the operating point of the baroreflex and its sensitivity. Preliminary data (on *H. malabaricus*, *H. unitaeniatus* and *O. niloticus*) suggests that exclusively aquatic fish have lower baroreflex sensitivity compared to amphibious fish - and also present baroreflex operating points located in the upper part of the sigmoid, contrary to their position in amphibious fish.

A12.80 LONG-TERM CARDIAC INFLUENCES OF ANGIO II IN THE EEL *A. ANGUILLA*: ROLE OF THE NOS/NO SYSTEM

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Angiotensin II (AngII), the active end-product of the Renin-Angiotensin System (RAS), plays important roles in the regulation of cardiovascular homeostasis and fluid osmolarity. In the eel *A. anguilla*, if administered for a month, AngII improves the performance of the heart, enhancing its ability to sustain increased afterload. These effects involve the AT₂ receptor, and factors which regulate cell growth and apoptosis (Imbrogno et al., 2013. *Gen. Comp. Endocrinol.* 194, 189-197). To deeper investigate the morpho-functional chronic influences of AngII on the eel heart and the molecular mechanisms involved, freshwater eels (*A. anguilla*) were intraperitoneally injected for 2 months with AngII (1 nmol/kg BW⁻¹). The hearts were isolated and subjected to morphological and western blotting analyses, and nitrite measurements. If compared to control animals, the ventricle of AngII-treated hearts showed an increase in compacta thickness, vascularization, muscle mass and fibrosis. Structural changes were paralleled by a higher expression of AT₂ receptor and a negative modulation of the ERK₁₋₂ pathway, together with a decrease in nitrite concentration, indicative of a reduced Nitric Oxide Synthase (NOS)-dependent NO production. Moreover, immunolocalization revealed, particularly on the endocardial endothelium (EE) of AngII-treated hearts, a significant reduction of phosphorylated NOS detected by p-eNOS antibody accompanied by an increased expression of the eNOS disabling protein NOSTRIN, and a decreased expression of the positive regulators of NOS activity, pAkt and Hsp90. Results suggest that AngII, by influencing the molecular mechanisms that control NOS activity and the ERK₁₋₂ pathway, induces structural and molecular remodelling of the eel heart.

A12.81 BARORECEPTOR LOCATION IN FISH WITH UNIMODAL AND BIMODAL RESPIRATION

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The barostatic reflex is one of the most important blood pressure regulating mechanisms that enables stable perfusion pressures and cardiovascular homeostasis in vertebrates. There is ample evidence that heart rate in teleost fish responds reciprocally to blood pressure, and while baroreceptors have been located in the gills, it remains uncertain whether receptors at other locations are involved. Furthermore, there is virtually no information on blood pressure regulation in air-breathing fishes. The present study, therefore, was designed to investigate the localization of baroreceptors in two closely related fish belonging to the Erythrinidae family, where *Hoplias malabaricus* is an exclusively water breathing species, while *Hoplerhythrinus unitaeniatus* is a facultative air-breather that uses the swim bladder for gas exchange. We choose trout (*Oncorhynchus mykiss*) as an outgroup because its cardiorespiratory responses are well-known. To characterize the cardiac limb of the barostatic reflex, heart rate was derived from the electrocardiogram before and after intraperitoneal infusions of the alpha-adrenergic agonist phenylephrine (100 µg.kg⁻¹) to cause vasoconstriction and the vasodilating nitric oxide donor sodium nitroprusside (250 µg.kg⁻¹). Both drugs were infused through a cannula inserted into the peritoneal cavity through a small cutaneous incision under anaesthesia on the previous day. To locate the baroreceptors responsible for these responses, the pharmacological manipulations of blood pressure were repeated after denervation (G4 group) of all branchial nerves. Preliminary results reveal that barostatic reflexes were absent in the G4 group in *H. malabaricus*, which demonstrates that baroreceptors are exclusively located in the gill arches in this species.

A12.82 VENTRICULAR Ca^{2+} CYCLING AND CONTRACTILITY IN HYPOXIA-ACCLIMATED ALASKA BLACKFISH (*DALLIA PECTORALIS*)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

● HOLLY A SHIELS (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), ED WHITE (UNIVERSITY OF LEEDS, UNITED KINGDOM), CHRISTINE COUTURIER (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES), DIARMID HALL (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES), SHANNON ROYAL (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES), GINA LJ GALLI (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), JONATHAN STECYK (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES)

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Few vertebrates have the capability to survive months in hypoxia. We investigated ventricular Ca^{2+} cycling and contractility of one such species, the Alaskan blackfish (*Dallia pectoralis*). Isolated ventricular myocytes loaded with Fura-2 and isometrically contracting ventricular strips from 5°C fish acclimated (5-8 weeks) to normoxia or hypoxia (10-20% air saturation; no air access) were stimulated to contract (0.2-0.6 Hz) during exposure to normoxic, hypoxic (20 min; 10% air saturation) and reoxygenated perfusate at 5°C and the impact on intracellular Ca^{2+} transients ($\Delta[Ca^{2+}]_i$) and contractile parameters assessed. The $\Delta[Ca^{2+}]_i$ and contraction kinetics from normoxic animals in normoxia were very slow. Ca^{2+} rise time, time-to-peak force (T_{PF}), Ca^{2+} 50% decay time and time-to-50% relaxation ($T_{0.5R}$) were 909 ± 135 , 984 ± 75 , 707 ± 83 and 647 ± 83 ms, respectively. Hypoxia acclimation did not affect $\Delta[Ca^{2+}]_i$ amplitude or maximal contractile force (F_{max}), but further slowed T_{PF} (by 30%) and almost doubled $T_{0.5R}$. Acute hypoxia did not dramatically affect the kinetics or amplitude of $\Delta[Ca^{2+}]_i$ or T_{PF} and $T_{0.5R}$ of both acclimation groups, whereas F_{max} was decreased, but by only ~50% in both groups. However, maximal attainable contraction frequencies were lower in hypoxia-acclimated myocytes and strips than preparations from normoxic animals in hypoxia. Moreover, the inability of hypoxia-acclimated strips to contract at high frequency persisted upon reoxygenation. The findings suggest ventricular Ca^{2+} cycling and contractility are resistant to hypoxia at routine contraction frequency. However, there may be limits and consequences to heart rate elevation during hypoxia, which may impact cardiac output in vivo.

A12.83 DRAGONFLY LARVAE AS MODELS FOR INVESTIGATING BEHAVIOUR-PHYSIOLOGY SYNDROMES IN CHANGING ENVIRONMENTS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

● JOACIM NÅSLUND (UNIVERSITY OF SOUTH BOHEMIA IN ČESKÉ BUDĚJOVICE, CZECH REPUBLIC), DAVID Š BOUKAL (UNIVERSITY OF SOUTH BOHEMIA IN ČESKÉ BUDĚJOVICE, CZECH REPUBLIC)

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Intraspecific consistent individual differences (CID) in behaviour (i.e. animal personality) and physiology are a hot topic in organismal biology, with possible consequences scaling from individual life histories to community dynamics. Traits showing CID are commonly hypothesized to be co-selected to form syndromes of covarying traits, so that expression of e.g. physiological traits can be predicted from the behavioural traits of an individual. We show that larvae of the Southern hawker dragonfly (*Aeshna cyanea*) display consistent individual differences in their spontaneous activity rates and respiration rates (used as a proxy for metabolism) across ontogeny and two temperatures. Respiration rates of the larvae were positively influenced by body size and temperature, while activity rates were not. Relatively high repeatability in spontaneous activity, together with a moderate repeatability of respiration rate, suggests that *A. cyanea* is a suitable model for investigations of the role of intra-specific CIDs in the effects of climate change on aquatic ecosystems. Surprisingly, we found no relationship between activity rates and metabolism, which contradicts some of the currently recognized theory. These results indicate that the inference of physiological characteristics based on behavioural traits, and vice versa, is not universal.

A12.84 TWO-CURRENT CHOICE FLUMES FOR TESTING AVOIDANCE AND PREFERENCE IN AQUATIC ANIMALS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

● FREDRIK JUTFELT (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY), JOSEFIN SUNDIN (UPPSALA UNIVERSITY, SWEDEN), GRAHAM RABY (UNIV. OF WINDSOR WINDSOR, CANADA), ANNA-SARA KRÅNG GRÅNS (IVL SWEDISH ENVIRONMENTAL RESEARCH INSTITUTE, SWEDEN), TIMOTHY D CLARK (UNIV. OF TASMANIA CSIRO AGRICULTURE AND FOOD HOBART, AUSTRALIA)

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Aquatic chemical ecology is an important and growing research field that involves understanding how organisms perceive and respond to chemical cues in their environment. Research assessing the preference or avoidance of a water source containing specific chemical cues has increased in popularity in recent years, and a variety of methods have been described in the scientific literature. However, there is a clear absence of standardized methodology, which makes comparisons across studies difficult. Some methodological problems occurring in the literature include turbulent flows causing mixing of cues, inappropriate size of choice

arenas for the animals, short experiments with stressed animals, failure to report how observation- and researcher-biases were eliminated, general underreporting of methodological details, and underutilisation of collected data. We propose best-practice guidelines on how to build, test and use two-current choice flumes to measure the behavioural responses of aquatic animals to chemical cues. The guidelines include steps that can be taken to avoid problems commonly encountered when using two-current choice flumes. Our aim is to provide a set of standards that will ensure data quality, transparency, and replicability in future studies in this field.

A12.85 ANALYSIS OF THE EFFECT OF BODY GROWTH ON THE STRUCTURAL PLASTICITY IN THE EPITHELIAL NERVE NET OF THE CTENOPHORE *PLEUROBRACHIA PILEUS*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

AMY COURTNEY (UNIVERSITY COLLEGE DUBLIN, IRELAND), CIARAN HICKIE (UNIVERSITY COLLEGE DUBLIN, IRELAND), MADELINE LOWERY (UNIVERSITY COLLEGE DUBLIN, IRELAND), SARA LOLATTE (UNIVERSITY COLLEGE DUBLIN, IRELAND), JÉRÉMY LIEGEY (UNIVERSITY COLLEGE DUBLIN, IRELAND), MARK PICKERING (UNIVERSITY COLLEGE DUBLIN, IRELAND)

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The ctenophore, *Pleurobrachia pileus* has a decentralised polygonal neuronal network beneath the epithelial layer. This nerve net must undergo structural rearrangement as the animal grows. In order to complete this work we need to accurately measure overall body surface area and the characteristics of the polygonal shapes within the nerve net. The nervous system in fixed *P. pileus* specimens collected from the Irish sea was visualised using an anti- β -tubulin antibody. A novel semi-automated neural tracing package was developed using Matlab to extract polygon information. Overall surface area was estimated using axial measurements, assuming a spheroid shape. Polygon area was not correlated with body surface area (range: 181-646 mm²) in either the aboral region ($n=6$, $p=0.563$, $r^2=0.099$) or the mid-body region ($p=0.919$, $r^2=0.007$). Similarly, polygon eccentricity did not correlate with size (aboral: $p=1$, $r^2=0.001$; mid-body: $p=0.356$, $r^2=0.236$). This suggests that polygon morphology remains constant throughout growth; implying a process of polygon addition. However, the validity of this conclusion depends on the accuracy of body surface area estimation, the most likely source of error. 3D whole animal reconstruction using optical projection tomography (OPT) was used for more direct morphometry is a separate cohort of animals, and indicated an unpredictable pattern of overestimation of surface area by spheroid modelling. Therefore, our conclusions remain tentative pending paired OPT and nerve net imaging, which we will investigate in the future.

A12.86 AN OXYTOCIN RECEPTOR ANTAGONIST INHIBITS SOCIAL PREFERENCE IN ZEBRAFISH

WEDNESDAY 5 JULY, 2017 POSTER SESSION

JENNY LANDIN (UNIVERSITY OF GOTHENBURG DEPT OF PHARMACOLOGY, SWEDEN), PETRONELLA KETTUNEN (INSTITUTE OF NEUROSCIENCE AND PHYSIOLOGY, SWEDEN), DANIEL HOVEY (INSTITUTE OF NEUROSCIENCE AND PHYSIOLOGY, SWEDEN), ANNA ZETTERGREN (INSTITUTE OF NEUROSCIENCE AND PHYSIOLOGY, SWEDEN), LARS WESTBERG (INSTITUTE OF NEUROSCIENCE AND PHYSIOLOGY, SWEDEN)

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The zebrafish is a model system suitable for psychiatric research due to well established behavioural tests, ease of pharmacological treatment, and available mutated lines of genes involved in human syndromes and disorders. Due to their strong tendency to shoal, zebrafish serve as a good model to study social behaviour. Although the neuropeptide oxytocin is known to be crucial for social behaviours in mammals, the role of isotocin (the fish homolog of oxytocin) in modulating social behaviours in zebrafish, is not yet clear. Our aim: to investigate if an oxytocin receptor antagonist inhibits social behaviour in adult zebrafish. Methods: adult male and female zebrafish were intraperitoneally injected with either the oxytocin receptor antagonist L-368,899 or vehicle, and subsequently tested in a social preference paradigm as well as a shoal study. Results: zebrafish treated with the oxytocin receptor antagonist displayed a tendency to decreased social preference compared to fish injected with vehicle. Conclusions: results indicate that endogenous isotocin may be involved in social preference in zebrafish, and show promise for future explorations of the network underlying social behaviour in the zebrafish.

A12.87 SHOAL SIZE INFLUENCES CAPTURE VULNERABILITY IN A SIMULATED PASSIVE GEAR FISHERY

WEDNESDAY 5 JULY, 2017 POSTER SESSION

DAVIDE THAMBITHURAI (UNIVERSITY OF GLASGOW, UNITED KINGDOM), JACK P W HOLLINS (UNIVERSITY OF GLASGOW, UNITED KINGDOM), TRAVIS E VAN LEEUWEN (CAPE ELEUTHERA INSTITUTE, BAHAMAS), ANITA RÁCZ (UNIVERSITY OF GLASGOW, UNITED KINGDOM), JAN LINDSTRÖM (UNIVERSITY OF GLASGOW, UNITED KINGDOM), KEVIN J PARSONS (UNIVERSITY OF GLASGOW, UNITED KINGDOM), SHAUN KILLEN (UNIVERSITY OF GLASGOW, UNITED KINGDOM)

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Human induced perturbations and harvest dominate contemporary evolution. A stark example of such change is reflected in wild fish populations where selective anthropogenic harvest far in excess of natural mortality has had profound evolutionary consequences. Our current understanding of how physiological and behavioural traits shape potential selection in fish is however very limited. High inter-individual variation in shoaling preference and metabolic rate has been recorded in fish and thus could potentially be a target for evolution, whether directly, or indirectly through correlated

selection. Here we study experimentally the effect of shoal size and metabolic rate on the vulnerability of wild zebrafish (*Danio rerio*) to trap capture. By testing fish in different shoal sizes and measuring their metabolic phenotype we show that fish in groups are significantly more vulnerable to capture than fish tested individually or at smaller group sizes. The results suggest that group behaviour, which is typically considered to be evolutionarily beneficial, may under a trap harvest scenario prove maladaptive. Contrary to our expectations metabolic variables show little or no relation with vulnerability to capture, with group size potentially overwhelming any potential modulation from metabolic phenotype. Together our findings are of broad interest for understanding some of the potential selective pathways of passive harvest fisheries and the physiological and behavioural mechanisms underpinning them.

A12.88 PRENATAL HYPOXIA CAUSES SEX-DEPENDENT ALTERATIONS TO MITOCHONDRIAL FUNCTION IN THE ADULT HEART

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Insufficient oxygen supply during development (prenatal hypoxia) has been linked to a range of cardiovascular abnormalities in adult life, including structural and functional changes to the heart. Nevertheless, the dysfunctional cellular pathways that underlie the altered cardiac phenotype remain largely unknown. As a highly oxidative organ, the heart is especially sensitive to hypoxia, due to its dependence on oxygen for mitochondrial ATP production. Therefore, we hypothesised that prenatal hypoxia during early life may permanently alter mitochondrial pathways and cause electron transport chain dysfunction in adulthood. Male and female C57 mice at 6 months of age were either exposed to normoxia during development, or hypoxia (14% oxygen) during gestational days 6 to 18. Mitochondrial function was assessed with an Oroboros microrespirometer. Prenatal hypoxia had sex-dependent effects on mitochondrial function. Females exposed to prenatal hypoxia had an overall reduction in reactive oxygen species production and an increase in routine respiration rate, suggesting an enhanced capacity to generate ATP. Males, on the other hand, show an increase in ROS production and no change in respiration. The mechanism for these changes is currently under investigation, but preliminary data indicate prenatal hypoxia causes sex-dependent metabolic changes to cardiac mitochondria which may predispose (males) or protect (females) offspring to cardiovascular disease in later life.

A12.89 CARDIAC PLASTICITY OF THE GOLDEN GREY MULLET IN RESPONSE TO LOWERED OMEGA 3 LEVEL IN FOOD SOURCE IN A WARMING SEAWATER CONTEXT

WEDNESDAY 5 JULY, 2017 POSTER SESSION

MARIE VAGNER (UMR LIENSS, FRANCE), THOMAS LACOUÉ-LABARTHE (UMR LIENSS, FRANCE), NATHALIE IMBERT (UMR LIENSS, FRANCE), JOSE LUIS ZAMBONINO INFANTE (UMR LEMAR IFREMER, FRANCE), EMMANUEL DUBILLOT (UMR LIENSS, FRANCE), VALÉRIE HUET (UMR LIENSS, FRANCE), PATRICK QUAZUGUEL (UMR LEMAR IFREMER, FRANCE), HERVÉ LE DELLIOU (UMR LEMAR IFREMER, FRANCE), CHRISTEL LEFRANÇOIS (UMR LIENSS, FRANCE)

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Recently, we observed that high-temperature acclimation under a low-omega3 diet, as expected under global change, increases energy expenditure during swimming in the golden grey mullet, a fish of high ecological value in temperate coastal areas. Here we focus on the underlying mechanisms governing this metabolic response at the individual level, particularly on the cardiac function, a key factor determining the physiological performance of teleost fish. Mulletts, fed with two diets differing in their omega3 concentrations, were exposed to two different temperatures during four months. Then, their performances were measured (1) at the individual level by respirometry to determine the critical swimming speed, as well as the standard and active metabolic rates, (2) at the heart level in order to understand the functionality and the relative importance of trans-sarcolemmal (SL) calcium influx and sarcoplasmic reticulum (SR) calcium flux on the excitation-contraction coupling, and (3) at the transcriptomic level to investigate differential gene expression patterns in heart tissue among the conditions tested. The results suggest that the combination of a low-omega3 diet and high temperature induces a shift in cardiac functioning strategy, with a lower SR participation and a higher involvement of the extracellular calcium through adrenergic pathway. We hypothesize that these combined factors could have changed the omega3 content in the cardiomyocyte membranes, modifying their functionality and leading to the activation of the adrenergic receptors and/or inhibition of the SR receptors present on the membranous surface. The cardiac adaptive capacity of this species will be discussed in a changing environmental context.

A12.90 ENDOTHELIN-1 INDUCES BOTH SYSTEMIC AND PULMONARY VASOCONSTRICTION IN *PYTHON REGIUS*

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The peptide hormone endothelin-1 (ET-1) is one of the most potent vasoregulators known. In mammals, the primary response to ET-1 is a prolonged vasoconstriction mediated through ET_A-receptors, whereas ET_B-receptors seem to have a more complex role, where they either induce vasorelaxation through nitric oxide release, or vasoconstriction when located in the vascular smooth muscle tissue. ET_B-receptors also function as 'clearance receptors' removing circulating ET-1 via the lysosomal pathway. Less is known about ET-1 signaling in reptiles. In turtles, ET-1 causes systemic vasodilatation mediated through activation of ET_B-receptors. In alligators, ET-1 causes systemic vasodilatation followed by constriction, whilst the pulmonary arteries constrict through stimulation of ET_A-receptors. In the present study, we investigate ET-1 signaling in pythons (*Python regius*) using both fully recovered snakes and studies of isolated blood vessels in myographs. Myograph studies show that ET-1 (10^{-10} - 10^{-7} M) leads to prolonged dose-dependent vasoconstriction in both mesenteric and pulmonary arteries. We found that the mesenteric vasoconstriction was attenuated after specific blockage of both ET_A and ET_B-receptors, respectively, using ET_A-antagonist BQ-123 (3×10^{-6} M) and ET_B-antagonist BQ-788 (3×10^{-6} M). The general ET-receptor antagonist bosentan (10^{-5} M) did not reduce the response further. In vivo studies on instrumented, recovered *Python regius* show that bolus intraarterial injections of ET-1 (120 pmol/kg) causes a major rise in systemic blood pressure that lasts for at least 1.5 hours. Injections of ET_A-receptor antagonists BQ-123 (0.15 μmol/kg) attenuated this pressor response by 55 ± 12%. It was however not possible to abolish the response completely.

A12.91 EFFECTS OF ACUTE HEAT STRESS ON HSP70 MRNA AND PROTEIN EXPRESSION IN ADULT ZEBRAFISH (*DANIO RERIO*)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Virtually all organisms respond to acutely increasing temperature (heat shock) by expression of heat shock proteins. The expression of heat shock genes in fish has usually been taken to be transcriptionally regulated, whereby *hsp* mRNA and protein change in concert. We have earlier found that the mRNA and protein production need not be coupled in a stenothermal Arctic salmonid, the Arctic char. To find out, if this is a peculiar property of Arctic fish, studies are also needed on species naturally inhabiting tropical areas. Therefore, the aim of this study is to investigate the effect of acute temperature stress on the expression and transduction of *hsp70* mRNA and protein level in a population of adult zebrafish (*Danio rerio*). For this purpose, 90 zebrafish were maintained at 26°C and exposed to three different stress temperatures (26, 31, 33°C) for 30 min, white muscle sampled at 3 different timepoints after the stress (0, 2, 24h) and rt-PCR and WB were performed in order to investigate the quantity of HSP70 mRNA and protein levels.

A12.92 ANAESTHESIA IN BALL PYTHONS: LOCATION MATTERS WHEN INJECTING ALFAXALONE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The synthetic neuroactive steroid alfaxalone provides safe, rapid anaesthesia in vertebrates, and unlike most other injectable anaesthetics, can be administered intramuscularly, which enables easy sedation in animals where vascular access is challenging. This study demonstrates the first use of intramuscular alfaxalone in snakes, where we identify the minimum required dose required to provide sufficient sedation for intubation for mechanical ventilation, to provide surgical anaesthesia with inhalant agents. Six ball pythons (*Python regius*) were each administered three doses (10, 20 and 30 mg/kg) of alfaxalone and anaesthetic induction and recovery were evaluated by assessing muscle tone, righting reflex, response to painful stimulation and ability to intubate. When injected into a caudal region of the body, 30 mg/kg was found to be the most effective dose, with 100% intubation success and rapid induction. As this is a higher dose than that recommended for other reptile species, the effect of injection site was also examined. The same six pythons were administered 20 mg/kg at a location cranial to the heart, and both speed and quality of induction were dramatically improved.

Even with 20 mg/kg, it was possible to intubate all six snakes, whilst only one could be intubated when alfaxalone was injected caudally. Total anaesthetic time also increased from 35 to 88 minutes when the injection site was moved. This study indicates that injection site has a marked influence on alfaxalone induction in snakes, and has stimulated further studies investigating the pharmacokinetics of this drug to explain these stark differences.

A12.93 THE EFFECTS OF NATURAL SEASONAL ACCLIMATIZATION AND ARTIFICIAL ACCLIMATION ON CARDIAC ELECTRICAL ACTIVITY IN EUROPEAN SCULPIN (*MYOXOCEPHALUS SCORPIUS*)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Ectothermic animals of high latitudes face great temperature changes during a year. Some of them are able to adjust their physiological functions to low temperatures. Most of the studies of thermal acclimation are based on artificially modelled seasonal acclimation. Some of the recent works focus on acclimatization in natural conditions and suggest that its effects are much stronger. The aim of the present study was to investigate and compare the effects of natural acclimatization (winter) and artificial seasonal acclimation (cold) on action potentials (APs) configuration and ionic currents in myocardium of European sculpin (*Myoxocephalus scorpius*). APs and ionic currents were recorded using standard whole-cell patch-clamp method from enzymatically isolated cardiomyocytes of sculpin. All experiments were approved by local bioethical committee. Significant decrease in AP duration on the levels of 50% and 90% repolarization was found in ventricular myocytes from winter-acclimatized (WA) animals if compared to cells from summer warm-acclimated (SA) ones; no differences were found between SA and summer cold-acclimated (CA) animals. Repolarizing currents I_{K1} and I_{Kr} were significantly upregulated in atrial and ventricular myocytes from WA animals compared to SA and CA groups. Calcium L-type current was significantly higher in atrial and ventricular myocytes of WA animals if compared to SA and CA. Sodium current was significantly downregulated in atrial and ventricular myocytes both in WA and CA groups if compared to SA. Thus, winter acclimatization in natural conditions greatly alters electrical activity of sculpin heart, while artificial cold acclimation of summer fish fails to do the same.

A12.94 THE EFFECT OF TEMPERATURE ON METABOLIC TRAITS AND WITHIN-SCHOOL POSITIONAL PREFERENCE OF EUROPEAN MINNOWS (*PHOXINUS PHOXINUS*)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Group living has a variety of costs and benefits, but these are not always distributed equally among individuals within a group. Individuals toward the front of groups may have better access to food but may be more vulnerable to predation. Furthermore, the spatial position that individuals occupy may be related to individual characteristics such as food demand or aerobic capacity. In swimming fish schools, individuals with higher metabolic demands or that are better swimmers may be located near the front of groups. However, we know little about how environmental factors such as temperature modulate links between positional preference within fish schools and physiological traits. Here we examine how acclimation temperature affects metabolic traits in juvenile European minnows (*Phoxinus phoxinus*) and in turn how this affects their positional preference within a swimming school. Sixty-three groups of 10 wild-caught minnows each were acclimated to three temperatures (16°C, 19°C, 22°C) and the positional preference (rank within the swimming school) of one focal fish per group was recorded in a swim tunnel at two different speeds (3 cm s⁻¹, 6 cm s⁻¹). Metabolic traits (standard and maximum metabolic rates) of focal individuals within each group were then estimated using intermittent flow respirometry. Analyses examine whether there is any modulating effect of temperature on the link between metabolic traits and positional preference within a group. The results from this study will increase our understanding of how the link between fish social behaviour and physiology may be affected by fluctuating temperatures.

A12.95 THE EFFECT METABOLIC RATE ON CHOICE OF GROUP SIZE IN A GREGARIOUS CORAL REEF FISH

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Group living comes with a variety of tradeoffs associated with food acquisition and predator avoidance. Individuals in larger groups may experience less predation risk but greater intraspecific competition for food resources. In addition, individual animals within a species vary widely in metabolic requirements, such that some individuals must feed more than others. A possible outcome is that individuals with a higher metabolic rate might be less social, or prefer to associate with smaller groups of conspecifics as the costs of competition may outweigh the benefits of predator avoidance. We studied these issues in redbelly yellowtail fusilier, *Caesio cuning*, a gregarious coral reef fish. The metabolic rate of all individuals was estimated via intermittent flow respirometry. Fish were then tested for sociability in a rectangular arena, where they were given a choice between associating with groups of various sizes (2 fish versus 5 fish; 2 fish versus 0 fish; and 5 fish versus 0 fish). All fish were tested under all three experimental conditions. Trials were recorded and analysed using automated tracking software to determine the amount of time in proximity to each shoal size. Early analyses suggest that fish with a higher SMR prefer smaller groups, most likely due to a higher prioritisation of foraging and a reduction in competition. This effect may also interact with baseline sociability as opposed to preference for shoal of a particular size *per se*. These results provide insight into the costs and benefits of group behaviours and the mechanistic underpinnings of sociability and group formation.

A12.96 Na^+/K^+ -ATPASE ACTIVITY IN THE ANOXIC TURTLE (*TRACHEMYS SCRIPTA*) BRAIN AT DIFFERENT ACCLIMATION TEMPERATURE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Survival of prolonged anoxia requires a balance between cellular ATP demand and anaerobic ATP supply from glycolysis, especially in critical tissues such as the brain. To add insight into the ATP demand of the brain of the anoxia-tolerant red-eared slider turtle (*Trachemys scripta*) during prolonged periods of anoxic submergence, we quantified and compared the number of Na^+/K^+ -ATPase units and their molecular activity in brain tissue from turtles acclimated to either 21°C or 5°C and exposed to either normoxia or anoxia (6h 21°C; 14d at 5°C). Na^+/K^+ -ATPase activity and density per g tissue were similar at 21°C and 5°C in normoxic turtles. Likewise, anoxia exposure at 21°C did not induce any change in Na^+/K^+ -ATPase activity or density. In contrast, prolonged anoxia at 5°C significantly reduced Na^+/K^+ -ATPase activity by 55%, which was largely driven by a 50% reduction of the number of Na^+/K^+ -ATPase units without a change in the activity of existing Na^+/K^+ -ATPase pumps or α -subunit composition. These findings are consistent with the "channel arrest" hypothesis to reduce turtle brain Na^+/K^+ -ATPase activity during prolonged, but not short-term anoxia, a change that likely helps them overwinter under low temperature, anoxic conditions.

A12.97 EFFECT OF ECOLOGICAL FACTORS ON GROWTH AND DEVELOPMENT OF THE DAUBED SHANNY *LEPTOCLINUS MACULATUS* POSTLARVAE FROM KONGSFJORD AND BILLEFJORD (SVALBARD)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Leptoclinus maculatus is a demersal fish that has a long multi-stage period of early development in the pelagic under the changing Arctic conditions (low temperatures, changes of water masses, photoperiod, food availability et al.). General and specific characteristics of growth and development of the daubed shanny postlarvae (pelagic and bottom) and their provision organ - «lipid sac» were studied. The research was made using of the Equipment Sharing Centre of the IB KarRCRAS. It was shown the linear growth of the daubed shanny in early ontogeny. Many organisms are characterized by accelerated growth in the certain periods of their life. A marked increase in length was shown for pelagic postlarvae of L2 stage and is assumed connected with their transition to exogenous nutrition of zooplankton. The lipid sac is formed at L2 stage, reached its maximum size at L4* stage and resorbed when fish becomes juvenile (L5). The availability of adaptive facilities in early ontogeny reflects the ways of directional influence of ecological factors on morphophysiological and biochemical processes, which contributes to the formation of phenotypic variability. The research was made in the frame of the budgetary theme No. 0221-2014-0033 and was supported by The Presidium of RAS «Searching fundamental research for development of the Russian Arctic» (No. 0221-2016-0001); the international project «SpitsEco» (ES504895).

A12.98 LATERALIZATION OF PARASYMPATHETIC CARDIAC CONTROL IN A TELEOST FISH (*COLOSSOMA MACROPOMUM*)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

VICTOR H S BRAGA (SÃO PAULO STATE UNIVERSITY (UNESP), BRAZIL), VINICIUS A ARMELIN (FEDERAL UNIVERSITY OF SÃO CARLOS (UFSCAR), BRAZIL), MARIANA T TEIXEIRA (SÃO PAULO STATE UNIVERSITY (UNESP), BRAZIL), FRANCISCO T RANTIN (FEDERAL UNIVERSITY OF SÃO CARLOS (UFSCAR), BRAZIL), LUIZ H FLORINDO (SÃO PAULO STATE UNIVERSITY (UNESP), BRAZIL)

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For long time, possible functional differences between the right and left sides of the vertebrates' nervous system have intrigued researchers. Some evidence point to the existence of lateralization in both the central and peripheral nervous systems in several species of vertebrates. Considering that little is known about this matter in fishes, and that this is an important issue for the knowledge on the evolution of the nervous system in vertebrates, the present study sought to verify whether the cardiac parasympathetic modulation is lateralized in *Colossoma macropomum*. For this, fish were instrumented with a ventral aortic cannula and a buccal cannula to allow for measurements of arterial blood pressure/heart rate and intrarterial/intrabuccal pharmacological administrations - they were also divided into "Intact Group", "Right-Vagotomy Group", "Left-Vagotomy Group" and "Bilateral-Vagotomy Group". All animals were submitted to the following situations that triggers parasympathetic cardiac modulation: fright caused by a standardized hit in their experimental chamber, chemoreflex induced by an intrabuccal administration of sodium cyanide, and baroreflex induced by an intrarterial administration of phenylephrine and sodium nitroprusside. Our preliminary data suggests that the left vagus nerve plays a greater role in the cardiac modulation of these animals, as the "Left-Vagotomy Group" present higher heart rate/blood pressure and less pronounced cardiac responses to the studied stimuli when compared to the "Right-Vagotomy Group". Thus, although both right and left vagus nerve participate in the modulation of these cardiac adjustments, our results point to the existence of certain level of lateralization in cardiac parasympathetic control in teleosts.

A12.100 *TRACHEMYS SCRIPTA* HCN ISOFORMS DISPLAY DIFFERENTIAL AFFINITY FOR THE SECONDARY MESSENGER cAMP

WEDNESDAY 5 JULY, 2017 POSTER SESSION

CHRISTINE S COUTURIER (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES), JASON BURKHEAD (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES), TIMOTHY HINTERBERGER (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES), JONATHAN A W STECYK (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES)

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A resetting of the intrinsic cardiac pacemaker is a significant contributor to the bradycardia essential for anoxia tolerance in the turtle *Trachemys scripta*. The hyperpolarization-activated cyclic nucleotide-gated channel (HCN, pacemaker or I_f channel) is considered a key determinant of cardiac pacemaking. HCN channels are homo- or hetero-tetramers formed by HCN 1-4 subunits. In mammals, the biophysical properties of HCN channels, such as kinetics, voltage dependence and sensitivity to intracellular and extracellular modulators such as cyclic nucleotides cAMP and cGMP, are determined by the subunit composition. Therefore, a differential expression of HCN isoform with anoxia could contribute to the slowing of intrinsic heart rate. Our objective was to discover if *T. scripta* HCN isoforms exhibit differences in cAMP binding affinity. We cloned *T. scripta* HCN subunits and discovered a novel HCN³ isoform (HCN3b) containing an insert of 7 amino acids in the cyclic nucleotide binding domain (CNBD). Then, direct fluorescence polarization was performed to assay 8-fluo-cAMP (0.3 nM) affinity using constructs of the C-Linker and CNBD for each isoform (protein concentrations of 1 nM to 100 μ M). HCN4 showed the highest affinity ($K_D = 684$ nM), HCN3b the least ($K_D = 6256$ nM) and the others subunits were intermediate ($K_D = 1623, 2613, 4424$ nM for HCN2, HCN3a and HCN1, respectively). Our results support that a remodeling of HCN channel isoform expression with anoxia exposure could contribute to resetting intrinsic cardiac pacemaker rate via altered sensitivity to cyclic nucleotides, the proportion of which varies with anoxia in the turtle heart.

A12.101 CHANGES IN CHEMICAL PROFILE PREDICT INFECTION STATUS IN MARINE FISH

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Animal diseases pose a major threat to the sustainability of global food production. Diagnosis is especially difficult in aquaculture and infections are often not detected before serious symptoms and death occurs. In addition, current standard diagnostic tools (ELISAs, PCR) only test for the presence of previously described pathogens, are most often invasive, and only detect infection in each individual tested, and not the population as a whole. The aim of this project was to test whether infectious status could be inferred through changes in the chemical profile released from marine fish. This knowledge could be used to develop innovative water-based technologies to monitor disease outbreaks in aquaculture.

We compared the profile of chemicals released into water by juvenile turbot (*Scophthalmus maximus*) infected with *Vibrio* sp. bacteria with the chemical profiles of mock infected controls. We also compared cortisol release (as an indicator of stress) and change in gene expression between the two groups. In our experiment, differences in the chemical profile could be used to predict infection, with 76% of cases fish allocated to the correct treatment group. Additionally, expression of both immune genes and genes involved in metabolism were affected by infection. The next step of this work will be to correlate differences in gene expression and chemical profile, with the eventual aim of elucidating mechanisms through which infection causes changes in chemicals released by fish.

A12.102 EFFECT OF OIL EXPOSURE ON SOCIAL BEHAVIOUR

TUESDAY 4 JULY, 2017 POSTER SESSION

TIFFANY A ARMSTRONG (UNIVERSITY OF GLASGOW, UNITED KINGDOM), ALEXIS J KHURSIGARA (UNIVERSITY OF TEXAS, UNITED STATES), SHAUN S KILLEN (UNIVERSITY OF GLASGOW, UNITED KINGDOM), ANDREW J ESBAUGH (UNIVERSITY OF TEXAS, UNITED STATES), KEVIN J PARSONS (UNIVERSITY OF GLASGOW, UNITED KINGDOM)

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In 2010, an estimated 4.9 million barrels of oil were discharged into the Gulf of Mexico during the Deepwater Horizon oil spill. It was the largest oil spill in history. The exposure of fish to crude oil is known to negatively influence developing fish embryos, damage tissues and organs, result in osmoregulatory difficulties, alter brain function and metabolic rates, and impact swimming and feeding behaviours. To determine the effect of oil exposure on exploration and social behaviour we exposed groups of four Atlantic croaker to a high dose (2%) and low dose (0.7%), as well as no exposure, resulting in groups of either no treatment, mixed (one exposed, three not), and all exposed. We found that high doses of oil altered the activity of fish in an open field, with larger nearest neighbour distances and a greater distance from the edge than non-exposed fish. Interestingly, mixed groups were effected more strongly than groups where all fish were treated. These results suggest that acute oil exposure can have an impact on social behaviour in shoaling species. Additionally, a single exposed fish may have a greater influence on shoal behaviour than when all fish are equally exposed.

A12.103 LIPID AND FATTY ACIDS CHANGES ASSOCIATED WITH THE SMOLTIFICATION OF SALMONID FISHES

TUESDAY 4 JULY, 2017 POSTER SESSION

SVETLANA A MURZINA (INSTITUTE OF BIOLOGY KARELIAN RESEARCH CENTRE OF THE RUSSIAN ACADEMY OF SCIENCES, RUSSIA), ZINAIDA A NEFEDOVA (INSTITUTE OF BIOLOGY KARELIAN RESEARCH CENTRE OF THE RUSSIAN ACADEMY OF SCIENCES, RUSSIA), SVETLANA N PEKKOEVA (INSTITUTE OF BIOLOGY KARELIAN RESEARCH CENTRE OF THE RUSSIAN ACADEMY OF SCIENCES, RUSSIA), NINA N NEMOVA (INSTITUTE OF BIOLOGY KARELIAN RESEARCH CENTRE OF THE RUSSIAN ACADEMY OF SCIENCES, RUSSIA)

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Smoltification is one of the main periods, characterized by alterations in metabolism, and physiological, morphological and behavioral changes, in the complex life cycle of salmonids through which juveniles inhabiting freshwater river sites prepare to move to the sea - new environment. Populations of Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) are abundant and have a reproduction in rivers of the White Sea Basin (Kola Peninsula). We studied the changes in the parameters of lipid metabolism: total lipid content, certain lipid classes (14 lipids) and fatty acids (FAs) profile of smolts of salmon and trout during smoltification using

Shared Equipment Centre IB KarRC RAS. The elevated level of biochemical metabolism, high hormone level (additional studies) of the smolts provides them with physiological readiness for transition to the marine environment. Certain lipid parameters and FAs ratios can be considered as indicators: the ratios of cholesterol to total phospholipids (PLs), and PLs to triacylglycerols, and the ratio of saturated FAs to polyunsaturated FAs, 16:0/18:1 ω -9, 18:2 ω -6/18:3 ω -3, 22:6 ω -3/18:3 ω -3, 20:4 ω -6/18:2 ω -6. Obtained results clearly define the changes in the synthesis of energy or structural lipids and demonstrate diverse and complex mechanisms of adaptation to change the environment (sea water). Determined differences in the biochemical status of juveniles may identify the begging and length of the smoltification, respectively, the time of migration to the sea, which in general is the basis for formation of the heterogeneous age structure of the population. The research was supported by the Russian Scientific Foundation, project no. 14-24-00102.

A12.104 TESTING THE OXYGEN HYPOTHESIS OF POLAR GIGANTISM: CUE THE AMPHIPODS

TUESDAY 4 JULY, 2017 POSTER SESSION

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High oxygen availability and its link to polar gigantism has attracted considerable attention particularly since Chapelle & Peck's (1999 Nature 399, 114-5) study concluded that 'maximum potential size is limited by oxygen availability'. One of the predictions of this 'oxygen hypothesis of polar gigantism' is that reductions in environmental oxygen tensions should affect larger-bodied animals more than small-bodied animals. Woods et al. (2009: Proc Roy Soc 276B:1069-75) found no evidence for any interaction between size and experimentally reduced oxygen tensions on sea spider performance, providing little support for the hypothesis. Consequently we have investigated the effects of hypoxia on aspects of the respiratory performance of a number of Antarctic amphipod species, spanning a wide range of body sizes. Some of these data will be presented and the level of support they provide for the 'oxygen hypothesis' discussed.

A12.105 FUNCTIONS OF INTERNAL MELANIN COLORATION IN ANURANS AND FISHES

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

👤 LILIAN FRANCO-BELUSSI (SAO PAULO STATE UNIVERSITY, BRAZIL), CLASSIUS DE OLIVEIRA (SAO PAULO STATE UNIVERSITY, BRAZIL)

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Fish and amphibians have in addition to colourful skin also pigmented cells on internal organs and membranes. Two cells types are observed in internal organs: melanocytes and melanomacrophages. Melanocytes are similar to dermal melanocytes and they found on the surface of various organs and connective tissue. In contrast, melanomacrophages occur in hematopoietic organs and have phagocytic activity similar to macrophages. Both melanocytes and melanomacrophages produce and store melanin, however melanomacrophages also has catabolic substances (e.g. hemosiderin and lipofuscin). Internal melanocytes occur differentially between species, and seem to exhibit phylogenetic characteristics in some anuran lineages. Although there are some evidences that this visceral pigmentation may be related to accumulation of residual melanin and may have a role in the innate immune system, the functions of visceral pigmentation in fish and anurans are largely unknown, antioxidant functions, and protect tissues against DNA damage. Now we will test the potential functions of visceral pigmentation in protecting internal organs of anurans and fish against UV damage. Specifically, we will measure DNA damage and cell death in two lineages of pigmented cells; melanocytes and melanomacrophages. Furthermore, possible functions related to innate immunity will be evaluated by measuring phagocytosis and production of melanin after exposure to bacteria.

A12.106 LOCALISATION AND CHARACTERISATION OF DORSAL HORN SPINAL NEURONS IN THE WISTAR RAT AND THEIR RESPONSE TO SACRAL NEUROMODULATION

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

👤 KEIRA TURNER (SCHOOL OF MEDICINE UNIVERSITY COLLEGE DUBLIN, IRELAND), RONAN O'CONNELL (CENTRE FOR COLORECTAL DISEASE ST VINCENT'S UNIVERSITY HOSPITAL DUBLIN, IRELAND), JAMES FX JONES (SCHOOL OF MEDICINE UNIVERSITY COLLEGE DUBLIN, IRELAND)

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The aim of this research is to investigate the mechanism of action of sacral neuromodulation (SNM) for faecal incontinence in a rat model. As a first step the location and inputs of spinal dorsal horn neurons receiving anorectal sensory drive have been studied. All experiments were conducted in accordance with protocols approved by the animal ethics research committee in University College Dublin and licensed by the Health Product Regulatory Authority, Ireland. Female adult Wistar rats (n=6, 200-300g) were anaesthetized with intraperitoneal urethane (1.5 g/kg) following isoflurane (5%) induction. After a laminectomy of the L1 and L2 vertebrae, extracellular recordings were taken using a glass microelectrode filled with a 2g% Pontamine sky blue solution in 0.9% saline. The anal canal was stimulated at 1Hz (12V) and SNM was applied to the S1 spinal nerve (3-5V, 3Hz, 3 mins). Following conclusion of the experiment, spinal cords were harvested, fixed in 4% paraformaldehyde, cyrosectioned, and imaged. Results: In total, 16 units with anorectal input were recorded at the S1 segmental level in either lamina 3 or 4 of the dorsal horn. Ten of the 16 units were classified as wide dynamic range (WDR) neurons and 13 units received A input and 3 units C-fibre input. Convergent S1 input was observed in 3 units. SNM produced an increase in the discharge of 8 units and a decrease in 4 units. Future studies will attempt to characterise the transmitter(s) involved in modulating anorectal input to the spinal cord.

A12.107 THE AUTOMATIC DEVICE OF SCORPION VENOM MILKING VES4

📅 **WEDNESDAY 5 JULY, 2017** **POSTER SESSION**

👤 **MOUAD MKAMEL** (FACULTY OF SCIENCES BEN MSIK HASSAN II UNIVERSITY, MOROCCO), **OMAR TANANE** (FACULTY OF SCIENCES BEN MSIK HASSAN II UNIVERSITY, MOROCCO), **ANASS KETTANI** (FACULTY OF SCIENCES BEN MSIK HASSAN II UNIVERSITY, MOROCCO), **RACHID SAILE** (FACULTY OF SCIENCES BEN MSIK HASSAN II UNIVERSITY, MOROCCO)

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In this research we have developed a device relied upon to extract scorpion venom by using electric stimulus across the muscles of the venom gland then droplets of venom are secreted from the aculeus. The automatic extractor of scorpion venom VES4® is specifically used for this purpose; it can be used in laboratory or in the field. This article describes the components of the VES4® also how to program it and how to operate it with a simple remote control. The electronic device was tested efficiently on four species of scorpions that were bred in the laboratory while respecting the vital parameters. The VES4® is designed to be a fast and secure instrument for the experimenter and scorpion it could also be required for more than four species.

A12.108 PHYSIOLOGICAL MECHANISMS BEHIND INTESTINAL L-LYSINE ABSORPTION IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

📅 **WEDNESDAY 5 JULY, 2017** **POSTER SESSION**

👤 **IDA HEDÉN** (UNIVERSITY OF GOTHENBURG DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES, SWEDEN), **HENRIK SUNDH** (UNIVERSITY OF GOTHENBURG DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES, SWEDEN), **ELISABETH J BERGMAN** (UNIVERSITY OF GOTHENBURG DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES, SWEDEN), **KRISTINA S SUNDELL** (UNIVERSITY OF GOTHENBURG DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES, SWEDEN)

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The intestine is a key organ for nutrient uptake and osmoregulation in fish. Na⁺/K⁺-ATPases (NKA) in the basolateral membrane of the enterocytes create a sodium gradient that acts as a main driving force for ion-coupled fluid and nutrient transport. Previous studies show that rainbow trout have higher L-lysine absorption in freshwater (FW) than seawater (SW). We hypothesized that there is a trade-off between ion-driven fluid and nutrient transport, i.e., that SW fish allocate more of the sodium gradient for fluid uptake at the cost of amino acid (AA) transport. The aim of this study was to clarify if intestinal L-lysine absorption in rainbow trout is sodium-dependent, and if inhibition of ion-driven fluid uptake in SW would increase the L-lysine uptake. Intestines from FW and SW adapted rainbow trout were mounted in Ussing chambers and L-lysine transport was assessed. Known inhibitors of primary and secondary transports: Ouabain; NKA, Bumetanide; NKCC and Hydrochlorothiazide; NCC, were added. Intestinal NKA activity was also measured and found to be higher in SW compared to FW fish. Inhibition of NKA and primary active transport resulted in a significant decrease in L-lysine transport in both FW- and SW-acclimated fish. Inhibition of NKCC2 and NCC did not affect the L-lysine transport. In conclusion, this study shows that L-lysine transport is largely sodium dependent and that the sodium gradient is increased after SW acclimation, but it cannot be confirmed that the lower L-Lysine uptake in SW is a result of a trade-off between intestinal ion-driven fluid and AA uptake.

A13 OPEN ANIMAL BIOLOGY

ORGANISED BY: CRAIG FRANKLIN (ANIMAL SECTION CHAIR, SEB)

A13.1 REASSESSING THE EFFECTS OF OCEAN ACIDIFICATION ON FISHES USING ROBUST AND TRANSPARENT APPROACHES

WEDNESDAY 5 JULY, 2017 09:00

TIMOTHY D CLARK (UNIVERSITY OF TASMANIA AND CSIRO HOBART, AUSTRALIA), FREDRIK JUTFELT (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY), DOMINIQUE G ROCHE (UNIVERSITÉ DE NEUCHÂTEL, SWITZERLAND), GRAHAM D RABY (UNIVERSITY OF WINDSOR, CANADA), SANDRA A BINNING (UNIVERSITÉ DE NEUCHÂTEL, SWITZERLAND), BEN SPEERS-ROESCH (UNIVERSITY OF NEW BRUNSWICK, CANADA), MIRJAM AMCOFF (STOCKHOLM UNIVERSITY, SWEDEN), FERNANDO MATEOS-GONZALEZ (UNIVERSITY OF TEXAS, UNITED STATES), JOSEFIN SUNDIN (UPPSALA UNIVERSITY, SWEDEN)

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Forecasted levels of CO₂ and ocean acidification over the next century have been reported to dramatically compromise the behaviour of fishes, particularly those on coral reefs. Most of this information has stemmed from short-term experimental manipulations, yet in many cases the experimental approaches are unusual and there has been little effort to remove experimenter bias. Using >2500 coral reef fishes from different species and life stages, we sought to replicate the previously-reported behavioural impairments with an objective to investigate the underlying physiological mechanisms. Using end-of-century estimates of CO₂ (~1000 µatm) and robust and transparent approaches (e.g., continuous video monitoring and automated tracking software), we have been unable to replicate the reported behavioural impairments despite a multitude of studies across three years. Specifically, coral reef fishes acclimated to end-of-century CO₂ do not differ from control individuals (at ~400-500 µatm) in their (1) ability to recognise and avoid predator chemical cues, (2) activity levels, metabolism and growth rates, (3) behavioural lateralisation, (4) acute thermal tolerance, or (5) boldness and susceptibility to predation. These findings are surprising given that many of the most prominent studies documenting detrimental effects of CO₂ on fish behaviour report strikingly low variability and large effect sizes, which should maximise the probability of successful replication. Our inability to replicate the dramatic effects suggests that research group methodology is causal to the discrepancy, casting doubts on the general applicability of the phenomenon.

A13.2 TRANSCRIPTIONAL EFFECTS OF MICROPLASTICS EXPOSURE IN DEVELOPING ZEBRAFISH

WEDNESDAY 5 JULY, 2017 09:15

CHRISTOPHE M R LEMOINE (BRANDON UNIVERSITY, CANADA), BAILEY M KELLEHER (BRANDON UNIVERSITY, CANADA), BRYAN J CASSONE (BRANDON UNIVERSITY, CANADA)

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Microplastics are small plastic particles, less than 5mm, that are manufactured or the result of physical degradation of larger plastic debris. Microplastics pollution has received a lot of attention in the last decades given its prevalence worldwide and the repercussions this type of pollution could have on our environment. Recent surveys have established the omnipresence of microplastics in aquatic ecosystems, including freshwater lakes and rivers worldwide. Despite these recent discoveries, there is only limited information on the impact these particles may have on the freshwater vertebrate species. The purpose of the present study was to evaluate the physiological impact of microplastics on the early life stages of zebrafish. Larval zebrafish were exposed to 0, 5 and 20 mg/L of fluorescently labelled polyethylene microspheres for up to 14 days and analyzed for particle content, growth, and global gene expression by RNA sequencing. Over the course of the experiment, fish showed an accumulation of microplastics in their gastrointestinal tract in a concentration dependent manner, but these particles did not seem to be affecting the development and growth of the fish overall. Interestingly, our results from whole larvae transcriptomics suggest that exposure to these particles caused a rapid differential regulation of thousands of essential metabolic and cellular process genes within 2 days, but that these effects were only transient as they largely disappeared by 14 days. However as these large changes in gene expression happened at a critical period of development, further studies are warranted to assess long-term impact of this pervasive pollutant.

A13.3 IS THERE A TRADE-OFF BETWEEN PEAK PERFORMANCE AND PERFORMANCE BREADTH ACROSS TEMPERATURES FOR AEROBIC SCOPE IN TELEOST FISHES?

WEDNESDAY 5 JULY, 2017 09:30

JULIE J H NATI (UNIVERSITY OF GLASGOW INSTITUTE OF BIODIVERSITY ANIMAL HEALTH AND COMPARATIVE MEDICINE, UNITED KINGDOM), JAN LINDSTRÖM (UNIVERSITY OF GLASGOW INSTITUTE OF BIODIVERSITY ANIMAL HEALTH AND COMPARATIVE MEDICINE, UNITED KINGDOM), LEWIS G HALSEY (UNIVERSITY OF ROEHAMPTON DEPARTMENT OF LIFE SCIENCES, UNITED KINGDOM), SHAUN S KILLEN (UNIVERSITY OF GLASGOW INSTITUTE OF BIODIVERSITY ANIMAL HEALTH AND COMPARATIVE MEDICINE, UNITED KINGDOM)

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Environmental thermal conditions strongly influence physiological and behavioural traits in ectotherms. At the species level, different thermal phenotypes (thermal specialist and thermal generalist) may arise from eco-evolutionary adaptation under different thermal environmental regimes. The “jack of all trades is a master of none” hypothesis suggests that thermal specialist species might have a higher peak performance but could only perform over a reduced thermal range, whereas thermal generalist species could perform over a larger range of temperatures but to through the cost of a reduced peak performance. As a result any performance trait either related to growth, reproduction, locomotion or metabolism, species would experience a negative correlation between thermal peak and breadth performance according to their thermal phenotypes. We tested this trade-off theory between thermal peak and breadth for aerobic scope performance (AS), which stands for the whole-animal capacity available to carry out simultaneous oxygen-demanding processes (e.g. growth, locomotion, reproduction) above maintenance levels, across 28 fish species. By performing a phylogenetically-informed comparative analysis on collected literature data for AS, we found no link between thermal peak and breadth performance for AS. The results of this study indicate that underlying physiological machinery influencing and determining thermal tolerance may compensate and stop such a trade-off from appearing. Therefore due these flexible, labile functional links between peak and thermal breadth for AS, may suggest AS may not be constrained by evolutionary responses to environmental changes such as climate warming.

A13.4 EFFECTS OF TEMPERATURE AND HYPOXIA ON SMALL-SPOTTED CATSHARK, *SCYLIORHINUS CANICULA* METABOLISM DURING EARLY DEVELOPMENT

WEDNESDAY 5 JULY, 2017 09:45

SYAFIQ M MUSA (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), JAMES DUCKER (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), JOHN L FITZPATRICK (STOCKHOLM UNIVERSITY, SWEDEN), HOLLY A SHIELS (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

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Due to human activities, our oceans are becoming warmer and coastal areas are becoming more hypoxic. This may be a problem for animals that develop in the intertidal zone, as early life stages can be particularly vulnerable. We studied the effect of chronic warming (from 15°C to 20°C) and chronic hypoxia (from normoxia to 50% oxygen saturation) on metabolic rate of Small-spotted catshark, *Scyliorhinus canicula* from early development to hatch, to understand how this animal survives in its changing habitat. A total of 50 *S. canicula* egg cases were reared in different holding tanks with different climatic conditions (normoxia at 15°C, normoxia at 20°C, hypoxia at 15°C, hypoxia at 20°C). Oxygen consumption of *S. canicula* inside the egg cases and at hatch was measured using a custom built 350 mL respirometry chamber and intermittent stop-flow respirometry. Results show that warming increased both resting and maximum metabolic rate in *S. canicula* inside the egg cases and after hatching. Hypoxia depressed metabolic rate at both temperatures and across all life stages. Moreover, maximum metabolic rate increased during development whereas resting metabolic rate did not. This study increases our understanding of the effects of climate change on the metabolism of marine top predators during their development in the intertidal zone, and may help in shark conservation for coastal areas.

A13.6 TEMPERATURE IMPACTS ON TOGENY OF THE IMMUNE SYSTEM IN EUROPEAN EEL, *ANGUILLA ANGUILLA* LARVAE

WEDNESDAY 5 JULY, 2017 10:15

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In endotherms temperature is a major modulator of the immune systems and its reactivity. Additionally, little knowledge exists on the immune system of European eel, *Anguilla anguilla*, especially during early life history, as those stages have never been encountered in nature. Advancements in this field are vital to understand the still enigmatic natural ecology and the potential impact of ocean warming on this critically endangered species.

We developed a new molecular tool box to study the immune system in European eel, involving 19 immunity-related genes from both the innate and the adaptive arm. In order to understand the regular ontogeny of the immune system and the influence of temperature during this process we reared larvae in four different temperatures throughout their thermal tolerance range (16, 18, 20, 22 and 24°C). We studied the expression of immune genes from hatch to first-feeding stage and established gene expression patterns during regular ontogeny at the optimal temperature at 18°C. Temperature levels below and above this optimum influenced expression of *mhc2*, *il1β* and *c-type lysozyme*. Larvae did not survive at 24°C. At the lower end of the thermal spectrum (16°C) immune competency appeared reduced, whilst close to the upper thermal limit (22°C) larvae showed signs of thermal stress. This led to increased immune expression probably in response to damaged cells. Protection against pathogens is thus probably impaired at temperatures close to CT_{max} , which can influence survival and productivity in aquaculture as well as natural recruitment with respect to anticipated global and ocean warming.

A13.7 MITOCHONDRIAL ADAPTATIONS OF INTERTIDAL FISH TO SURVIVE HYPOXIA: A MULTIDIRECTIONAL APPROACH

WEDNESDAY 5 JULY, 2017 11:00

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Most of a cell's energetic requirements in ATP is supplied by mitochondrial (*mt*) oxidative phosphorylation (*oxphos*). During hypoxic exposure *oxphos* slows, and a cascade of physiological mechanisms may arrest life if metabolism is not appropriately adjusted. A switch to anaerobic metabolism alters cellular redox balance, greater ATP hydrolysis than formation, leads to lactate and associated proton accumulation with subsequent intracellular acidosis, all of which eventuate in dysregulation of the cell. Along with lactate, the neurotransmitter glutamate is released and accumulates in ischemic brain, which activates postsynaptic receptors, causing abnormal Ca^{2+} entry. While neurons actively buffer Ca^{2+} , excess Ca^{2+} can damage *mt* and promote apoptosis via *mt* cytochrome c release. Succinate also accumulates in ischemic brain and on reperfusion is associated with deleterious *mt* ROS production. We predicted that hypoxia tolerant species (HTS) such as rock-pool/intertidal fish should better tolerate acidosis, display greater glutamate clearance, have greater Ca^{2+} buffering capacities, and limit succinate-induced ROS production. Therefore, four New Zealand triplefin fish species (Family: Tripterygiidae) were compared, each with different hypoxia-tolerances, ranging from high intertidal to subtidal niches. Although Ca^{2+} buffering was similar among all fish species, subtidal species were more sensitive to lactate-induced acidosis, while HTS *mt* maintained *mt* function. Moreover, HTS showed greater glutamate clearance capacity, and lower *mt* ROS production. Overall, brain mitochondria from intertidal fish display a metabolic profile that likely aids survival during hypoxia exposure in the intertidal zone.

A13.8 HYPOXIC PRECONDITIONING PROTECTS BRAIN MITOCHONDRIA FROM RE-OXYGENATION INJURY

WEDNESDAY 5 JULY, 2017 11:15

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Diminished oxygen supply occurring during environmental challenge or physiological trauma is primary causes of ischemia/reperfusion (I/R) injury in the brain resulting in irreversible brain damage or death. Investigating and identifying the neuroprotective mechanisms used by two tropical animals with the capacity to tolerate several hours of hypoxia or anoxia followed by re-oxygenation could provide novel strategies for use in conservation and in clinical settings. The epaulette shark (*Hemiscyllium ocellatum*) and the grey carpet shark (*Chiloscyllium punctatum*) have evolved hypoxia/anoxia tolerance using different mechanisms: metabolic depression or the release of stored red blood cells, respectively. The effect of hypoxia preconditioning on mitochondrial respiration is unknown and was examined in cerebellar tissue from both species with or without prior exposure to 2hrs of hypoxic pre-conditioning in vivo, administered 24 hours prior to tissue collection. Animals were euthanized with benzocaine, the cerebellum removed, homogenized in cold MiRO5 and examined in an Oroboros respirometer. There were significant decreases in flux through oxidative phosphorylation (complex I and II) in both hypoxia preconditioned epaulette sharks and grey carpet sharks during the re-oxygenation phase in comparison to the level before anoxic challenge. This did not occur in normoxia treated grey carpet sharks. These data demonstrate that hypoxic preconditioning can have a protective effect during re-oxygenation even in species that do not naturally enter a phase of metabolic depression and could have relevance in species conservation and in clinical settings.

A13.9 DAILY CYCLIC HYPOXIA ACCELERATES THE MOULT CYCLE IN THE SHRIMP *PALAEEMON VARIANS* AND INDUCES MORPHOLOGIC CHANGES IN THE GILLS

WEDNESDAY 5 JULY, 2017 11:30

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In shallow-water areas hypoxia is increasing in frequency and is considered a major threat to biota, ultimately translating into changes in biodiversity and ecosystem functioning. To determine the ecophysiological implications of hypoxia we exposed a shallow-water model - the shrimp *Palaemon varians* - to oxygen fluctuations

down to species' critical oxygen partial pressure (p_{crit}) for 7 hour each night. We have identified significant changes in moult-related gene expression, which has never been described in crustaceans. To further validate changes in gene expression with an altered phenotype, we conducted experiments to determine changes in the duration of the moult cycle. Collectively, results clearly identify changes to the regulation and duration of the moult cycle, which is accelerated in response to hypoxia. Our data support earlier observations of how insects accelerate moult cycle and develop a larger tracheal system as a consequence of sustained hypoxia, and potentially indicate an evolutionarily conserved physiological response to environmental perturbation for terrestrial and marine phyla. Finally, in order to investigate the relation between a physiological constraint (hypoxia) and gill morphology in P. varians, we compared histological, morphometric and computed tomography analysis of gills of shrimp kept in normoxia and in cyclic hypoxia. Results showed that lamellar length and surface area are increased in shrimps kept in hypoxia, compared to shrimps in normoxia (p-values 0.029, 0.028, respectively). Results clearly underline the extent to which a physiological limitation triggers a morphological change in a shallow-water shrimp and identify the mechanism by which this takes place.

A13.10 INTRASPECIFIC VARIATION AND THERMAL ACCLIMATION EFFECTS ON MITOCHONDRIAL FUNCTION IN A EURYTHERMAL TELEOST (*FUNDULUS HETEROCLITUS*)

WEDNESDAY 5 JULY, 2017 13:50

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Whole-animal thermal limits and hypoxia tolerance are thought to be constrained by processes acting at the level of the mitochondrion. Understanding these mechanisms is becoming more important as anthropogenic climate change drives shifts in species' ranges. In the present experiments, we tested the effects and trade-offs of thermal acclimation on mitochondrial performance in locally adapted subspecies of the Atlantic killifish, *Fundulus heteroclitus*. We acclimated (5, 15, 33°C) northern and southern killifish and measured mitochondrial performance (respiratory capacity, O_2 binding affinity) and associated trade-offs (loss of mitochondrial membrane potential, increased ROS production). We demonstrate greater mitochondrial respiratory capacity in the northern subspecies and greater O_2 binding affinity in the southern subspecies. These data indicate a role for mitochondrial function in setting aerobic performance limits and putative targets of selection. Thermal acclimation was associated with large changes in mitochondrial respiration rate, particularly at 33°C. These shifts in performance occurred primarily through electron transport chain complex I and were not associated with large trade-offs in maintenance of membrane potential or ROS production perhaps accounting for the eurythermal physiology of this species. Our observations strongly support the probable importance of mitochondrial function in the setting of aerobic performance limits in ectotherms.

A13.11 DOES OXIDATIVE STRESS LIMIT COLD ACCLIMATION IN MOSQUITO FISH (*GAMBUSIA HOLBROOKI*)?

WEDNESDAY 5 JULY, 2017 14:05

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Thermal acclimation allows individuals to remodel their physiology to compensate for the negative effects of changing environmental temperatures on performance. There is considerable variation between individuals in the capacity for acclimation within natural populations, which implies that there may be costs that constrain its evolution. Oxidative stress, where the production of reactive oxygen species (ROS) exceeds antioxidant defences, may represent a cost of acclimation to colder temperatures due to increased mitochondrial respiration rates. Here, we used mosquito fish (*Gambusia holbrooki*) to test the hypothesis that inter-individual variation in the capacity for cold acclimation was mediated by variation in the susceptibility to oxidative damage. We found that neither acclimation temperature (3-4 weeks at 18°C or 28°C) nor increasing antioxidant capacity by administering N-acetyl-cysteine (NAC) affected ROS-induced protein damage. However, cold acclimated fish had significantly higher catalase antioxidant activity, and NAC reduced this increase in activity, suggesting that ROS production is higher during cold acclimation because catalase is induced by ROS. Catalase activity was higher in fish with a low acclimation capacity, suggesting that they produce more ROS than fish with a high acclimation capacity. Concurrently, mitochondrial coupling and ATP production rates were higher in cold acclimated fish. Acclimation of sustained swimming performance was incomplete, and NAC had no effect, indicating that cold acclimation of locomotor performance is not limited by ROS production or ATP supply. Therefore, increased oxidative damage was avoided by upregulating antioxidant activities during cold acclimation, however the energetic cost of maintaining oxidative balance may represent a cost of acclimation.

A13.12 DEVELOPMENTAL AND REVERSIBLE THERMAL PLASTICITY AFFECT DISPERSAL IN GUPPIES (*POECILIA RETICULATA*)

📅 WEDNESDAY 5 JULY, 2017 ⌚ 14:20

👤 AMÉLIE LE ROY (THE UNIVERSITY OF SYDNEY, AUSTRALIA), FRANK SEEBACHER (THE UNIVERSITY OF SYDNEY, AUSTRALIA)

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Evolutionary theory predicts that intergenerational environmental fluctuations should favour developmental modifiers that match offspring phenotype to their environment. However, in highly fluctuating environments, mismatches between parental and offspring environments occur, in which case reversible acclimation in offspring could alleviate the cost of developmental plasticity. Our aim was to determine whether reversible thermal acclimation reduces the cost of developmental thermal plasticity on performance and exploratory behaviour in guppies. We predicted that matching both developmental and acclimation temperatures to acute environmental temperature would maximize locomotor performance, and therefore increase exploratory behaviour and dispersal. Alternatively, when developmental temperature was mismatched to the acute environmental temperature, we predicted that thermal acclimation would alleviate this mismatch by shifting performance curves.

As hypothesised, locomotor performance (U_{crit}) was greatest when developmental, acclimation and acute test temperatures matched. When there was a mismatch between developmental and acute test temperatures, cold acclimation partially alleviated the performance cost of this mismatch. Interestingly, U_{crit} , representing maximal swimming capacity, was not correlated to exploration. We measured exploration in an 8 m channel interspersed by five pools. Developmental conditions did not affect exploration in females, but affected all exploration traits (time to leave first pool, voluntary movement speed, number of dispersal decisions) measured in males, which explored more than females. Acclimation affected exploration in both sexes.

Our results question the ecological relevance of maximal performance measures, and suggest that developmental and reversible thermal plasticity play a key role in determining dispersal rates, with potential consequences for meta-population dynamics, invasion rates and range shifting.

A13.13 TEMPERATURE EFFECTS ON BODY AND MUSCLE GROWTH IN TWO ECOTYPES OF WHITEFISH *COREGONUS LAVARETUS*

📅 WEDNESDAY 5 JULY, 2017 ⌚ 14:35

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Temperature is an important factor that influences teleost body and muscle growth. However, little is still known about the cellular processes behind this phenomenon. Here, data are presented that demonstrate that different thermal experiences in embryonic life aid the segregation of two phenotypically divergent forms of white fish *Coregonus lavaretus*. Batches of fish of a dwarf and a normal form were kept at 2° and 6° until hatching and subjected to similar thermal treatment afterwards. Results demonstrate clearly that fish of the regular form are much smaller when imprinted at thermal conditions typical for the spawning sites of the dwarf form (6°C) than when imprinted at the conditions usually experienced at their own spawning sites (2°C). Surprisingly, the fish of the dwarf form exhibit a similar response pattern to thermal history (2°-fish much larger than 6°-fish), indicating that in their case, normal spawning site temperature (6°C) is indeed likely to act as a growth limiting factor. Results also demonstrate that hypertrophic and hyperplastic muscle growth modes are similarly affected by temperature. Immunolabelling experiments for Pax7, H3P and MEF2 were performed to quantitatively examine muscle precursor cells including such that are mitotically active or have entered differentiation. Results demonstrate that incubation temperature has an important influence on the proliferation/differentiation balance of such cells in the two ecotypes.

A13.14 THE EFFECT OF STRESS ON IMMUNOCOMPETENCE OF BANK VOLES FROM A MULTIDIRECTIONAL SELECTION EXPERIMENT

WEDNESDAY 5 JULY, 2017 14:50

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One of topical problems in evolutionary physiology concerns proximate mechanisms limiting development of complex adaptations. A tradeoff-based argument implies that benefits achieved by selection for increased performance in one function are compromised by decreased performance in others. On the other hand, the improvement in one trait may open a possibility of positive changes in several others. We approached the problem using a unique experimental evolution model system - lines of bank voles (*Myodes glareolus*) selected in three directions: high aerobic exercise metabolism (A), predatory behaviour (P), and ability to cope with low-quality herbivorous diet (H), and unselected control lines (C). We asked whether immunocompetence, measured as antibodies level developed after injection of a non-pathogenic antigen (SRBC), has changed as a correlated response to the selection, and whether the hypothetical selection-related differences depend also on environmental conditions under which the immune response is mounted: a) comfortable exposure to b) thermoregulatory burden or c) acute restraint stress or d) a chronic mild stress. Preliminary results showed that the secondary humoral response (after second injection of SRBC) was stronger than the primary, but there were no effects of selection. Thermoregulatory burden (exposure to +5°C) resulted in decreased response (and also a decreased mass of thymus), but, surprisingly, exposure to the acute or chronic mild stress tended to increase the response (but the difference was not significant). No interaction between selection and stress-exposure was observed. Thus, large increases of the performance traits observed in the selected lines were not accompanied by compromised immunocompetence.

A13.19 HOW TO CATCH AN ANCHOVY: CHARACTERISTICS OF HUMPBACK WHALE LUNGES AND THE TIMING OF ANCHOVY ESCAPE

WEDNESDAY 5 JULY, 2017 15:45

NICHOLAS CAREY (HOPKINS MARINE STATION STANFORD UNIVERSITY, UNITED STATES), PAOLO DOMENICI (IAMC-CNR ISTITUTO PER L'AMBIENTE MARINO COSTIERO, ITALY), DAVID E CADE (HOPKINS MARINE STATION STANFORD UNIVERSITY, UNITED STATES), JEREMY A GOLDBOGEN (HOPKINS MARINE STATION STANFORD UNIVERSITY, UNITED STATES)

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Forage fish, such as anchovies and sardines, occur in large aggregations which are targeted by numerous marine predators using various attack strategies. One major predator of forage fish, the humpback whale (*Megaptera novaeangliae*), captures prey via high-speed, high-acceleration lunges, with the characteristics and timings of these lunges varying according to the species targeted. This suggests that to optimise capture success, these energetically expensive lunges are modified according to the prey's ability to escape. Prey escape responses are also energetically expensive, so are usually only initiated when a perceived threat is at a certain distance and approaching at a certain speed. We present a study which brings together data from whale lunge-feeding events on anchovies and krill, and experimental tests of the timing of anchovy escape responses. Speeds, acceleration, and gap timings of humpback whale lunges on either anchovies or krill were measured using biologging tags with on-board video and 3D accelerometry. In subsequent lab experiments, escape responses of Pacific anchovies (*Engraulis mordax*) were triggered by a looming shape on a screen simulating a whale approaching, based on various speed profiles. Escape responses were filmed using high-speed cameras and analysed for the timing, escape direction, and perceived distance of the threat. Our results illustrate the complex balance between whale attacks and anchovy escape responses, and how the lunge characteristics of the whales may be related to the escape abilities of targeted prey.

A13.20 A NON-LINEAR RELATIONSHIP BETWEEN SWIMMING METABOLISM AND SPEED IN A NEGATIVELY BUOYANT FISH

WEDNESDAY 5 JULY, 2017 16:00

VALENTINA DI SANTO (HARVARD UNIVERSITY, UNITED STATES), CHRISTOPHER P KENALEY (BOSTON COLLEGE, UNITED STATES), GEORGE V LAUDER (HARVARD UNIVERSITY, UNITED STATES)

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Swimming performance is considered a key trait determining the ability of a fish to find food, refugia, and mates, and to avoid unfavorable conditions and escape predators. Typically, metabolic rates increase with speed up to a critical point: the critical swimming speed at which fish fatigue. At the same time, fish must stabilize their body posture at very low speeds and thereby incur high energetic costs. The combination of high metabolic costs at extreme speeds and relatively lower costs at an intermediate cruising speed, may result in a non-linear metabolic-speed relationship. However, to date there are no complete data sets to confirm this hypothesis. In this study we quantified the metabolic costs associated with varying speed ($0.75\text{--}2.25 \text{ BL} \times \text{s}^{-1}$) in a negatively buoyant fish, the clearnose skate *Raja eglanteria*. We employed two approaches, a classic critical swimming speed protocol and a single-speed exercise and recovery procedure. We found a discrepancy in the metabolic-speed relationships using the two methods. When using the single-speeds approach we observed a J-shaped aerobic metabolic-speed relationship and an anaerobic component at each velocity tested. These results suggest that anaerobic metabolism is involved during low as well as high swimming speeds in the clearnose skate and that critical swimming protocols might misrepresent true costs of locomotion across speeds in negatively buoyant fishes.

A13.21 VERTICAL FLIGHT - A TRACTABLE METHOD FOR STUDYING ENERGY-ACCELEROMETRY RELATIONSHIPS IN BIRDS

WEDNESDAY 5 JULY, 2017 16:15

TESSA A VAN WALSUM (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM), LEWIS G HALSEY (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM), ANDREA PERNA (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM), PHILIP M COLLINS (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM), CHARLES M BISHOP (BANGOR UNIVERSITY, UNITED KINGDOM)

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While accelerometry sometimes correlates with rate of energy expenditure in terrestrial species, its suitability for volant animals has so far received little attention. This is in part because calibrating a proxy of energy expenditure during flight is particularly difficult. However, the external energy costs of gaining height during vertical flight can be easily calculated, providing the opportunity to compare this cost against measures of acceleration on a data logger instrumented to a trained bird. We calibrated dynamic body acceleration (DBA) - a derivation of acceleration measured on an animal-instrumented accelerometer - against the external power required to gain height in Harris' Hawks at the Hawk Conservancy

Trust in Andover. The hawks flew to 6 different heights (range: 0-4.1 m) with only a minimal horizontal distance covered (4.1 m). The flights were short - average flight duration was 2 s, during which the hawks typically flapped their wings 5 to 7 times. We found that the Hawks' external power increased linearly as the DBA of their centre of mass increased (preliminary analysis: $R^2=0.55$). If it is assumed that the relationship between power and DBA during near-vertical flight holds for horizontal flying then DBA could be used to estimate external power costs in the wild. Furthermore, assumptions about the ratio of external power costs to internal power costs could enable DBA to also predict total energy costs, i.e. metabolic rate, during flight.

A13.22 GUT MOTILITY - AN ESSENTIAL BUT UNDERAPPRECIATED ASPECT OF A MULTIFUNCTIONAL ORGAN

WEDNESDAY 5 JULY, 2017 16:15

CATHARINA OLSSON (DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES UNIVERSITY OF GOTHENBURG, SWEDEN), JEROEN BRIJS (DEPARTMENT OF ANIMAL ENVIRONMENT AND HEALTH SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), ALBIN GRÄNS (DEPARTMENT OF ANIMAL ENVIRONMENT AND HEALTH SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), GRANT HENNIG (DEPARTMENT OF PHARMACOLOGY UNIVERSITY OF VERMONT, UNITED STATES)

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Gut motility provides the fundament for proper performance of the gastrointestinal tract, in digestion and absorption of nutrients as well as in maintaining homeostasis and overall health. This is reflected in a variety of different motility patterns. In a series of studies we have described general motility patterns in fish, and here we summarize the main differences and similarities between two teleosts, with slightly different gut morphology and feeding habits, namely rainbow trout (*Oncorhynchus mykiss*) and short horn sculpin (*Myoxocephalus scorpius*). Intestinal motility of the two species was qualitatively and quantitatively described using spatio-temporal maps constructed from in vivo video recordings. Both species showed propagating and standing contraction of various strength, speed and frequencies. The predominating propagating contractions were shallow, relatively high frequency, high speed ripples and more slowly propagating contractions. The latter, at least in rainbow trout, showed clear resemblance with the mammalian migrating motor complexes (MMCs) that serve housekeeping functions in the gut. The contraction frequency in rainbow trout was generally lower than in sculpin, but increased when trout was transferred to seawater, indicating a role in osmoregulation as well. Furthermore, by combining video recordings and recordings of enteric electrical activity (EEA) in anaesthetized fish we have now verified the correlation between contractions and EEA. This opens up the possibility to use EEA recordings in conscious fish to study gut motility patterns in more details, including how they are affected by changes in environmental conditions such as temperature or salinity as well as by feeding.

A13.15 TRANSGENERATIONAL EPIGENETIC RESPONSE OF ZEBRAFISH (*DANIO RERIO*) EXPOSED TO PAHS: MOLECULAR, MATERNAL AND PATERNAL EFFECTS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

AMÉLIE CRESPEL (UNIVERSITY OF GLASGOW, UNITED KINGDOM), NAIM BAUTISTA (UNIVERSITY OF NORTH TEXAS, UNITED STATES), JANNA CROSSLEY (UNIVERSITY OF NORTH TEXAS, UNITED STATES), PAMELA PADILLA (UNIVERSITY OF NORTH TEXAS, UNITED STATES), WARREN BURGREN (UNIVERSITY OF NORTH TEXAS, UNITED STATES)

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Parental exposure to environmental stressors can have repercussions on offspring phenotype. Such effects modify the capacity of populations to cope with environmental stressors across generations. However, little is known about the mechanisms underlying the transgenerational response, such as the relative importance of maternal or paternal effects, in addition to the molecular epigenetic effects that can be involved. To address these issues, adult zebrafish (*Danio rerio*) were fed with 0% or 100% of crude oil extract (HEWAF) supplemented diet for 21 days. Four groups of offspring were then created: control group (parents fed 0% HEWAF), male exposed group (female fed 0% HEWAF, male fed 100% HEWAF), female exposed group (female fed 100% HEWAF, male 0% HEWAF), and exposed group (parents fed 100% HEWAF). The phenotypic response on the fitness and cardiac capacity of the parents and the offspring was then evaluated and tissues were sampled for DNA methylation analysis. No difference was observed in the parental phenotype. However, an enhanced tolerance to PAHs was observed in the offspring. At the molecular level, a decrease in the global DNA methylation was observed in the parents exposed to PAHs and in the offspring derived from one or both parents exposed to PAHs. This decrease was accompanied with an up-regulation of a gene (GNMT) involved in methylation transfer in the parents but not in the offspring. These results suggest that the modification of the DNA methylation might have been epigenetically inherited and that both parents influenced the molecular and phenotypic response of the offspring.

A13.16 REACTIVITY STUDIES OF FERRIC HAEMOGLOBIN REVEAL A POTENTIAL ROLE IN HYDROGEN SULFIDE (H₂S) SIGNALLING

WEDNESDAY 5 JULY, 2017 POSTER SESSION

BIRGITTE JENSEN (DEPARTMENT OF BIOSCIENCE AARHUS UNIVERSITY, DENMARK), ANGELA FAGO (DEPARTMENT OF BIOSCIENCE AARHUS UNIVERSITY, DENMARK)

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The ill-smelling gas hydrogen sulfide (H₂S) is a signalling molecule, contributing to several important physiological functions such as regulation of vascular tone and suppressed metabolism. The importance of its biological role and metabolism is still largely elusive. Even the *in vivo* concentration in tissues is still a matter of debate. Recently, a new heme-dependent oxidation of H₂S was discovered where the O₂ binding protein of blood, haemoglobin (Hb), in its ferric state Hb(Fe³⁺) can oxidize H₂S to thiosulfate (S₂O₃²⁻) and iron-bound hydropolysulfides (R-S-S_n-H), thus preventing accumulation of H₂S to toxic levels that will inhibit mitochondrial respiration. By applying spectrophotometric methods to study the kinetics and equilibrium of the heme-H₂S complex of human Hb(Fe³⁺), we found evidence for additional roles of this protein in relation to sulfide metabolism and signalling. During anaerobic conditions, we found a high affinity of Hb(Fe³⁺) for H₂S, which appeared to be pH-dependent. We speculate that Hb(Fe³⁺) can act as a physiological carrier of H₂S, whereby the small amount (approx. 1%) of Hb(Fe³⁺) in the red blood cells may be able to release H₂S to the surrounding tissues. This would imply a new possible role for ferric Hb, normally believed to be of little or no biological relevance. H₂S transport in the blood may help explain the profound physiological effects of H₂S in vertebrates, particularly in hibernating mammals, where a redistribution of sulfide pools may take place in the blood of hibernating brown bears during metabolic suppression.

A13.17 CAN BIO-LOGGERS BE USED TO ASSESS STRESS AND WELFARE OF FISH IN AQUACULTURE?

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

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High mortality rates of commercially farmed fish has been shown to be linked with acute and chronic stress levels, and thus research in stress physiology is of increasing interest. Since physiological stress responses of freely swimming farmed fish are sparse, we implanted 20 rainbow trout (*Oncorhynchus mykiss*) with data storage tags recording heart rate and released them into sea cages with 5000 conspecifics for 3 weeks. Following this period, the fish were subjected to a series of stressful events that normally occur during harvesting (i.e. crowding, netting, transport and CO₂ narcosis). To further quantify and validate these stress responses, a subsample (n=20 for each event) of uninstrumented fish were sampled for plasma cortisol. Crowding initiated a clear stress response as evident from elevated heart rate and plasma cortisol. Following crowding, the fish were netted onto a well-boat and transported for 60 min, during which heart rate and plasma cortisol continued to increase. The fish were then left to recover overnight (18 h) before common slaughter routines ensued the following day (i.e. crowding, netting and CO₂ narcosis). Heart rate and plasma cortisol levels were still elevated the following morning indicating that the fish had not yet fully recovered from the previous day. Furthermore, the fish were more sensitive to stressors as the mere presence of humans initiated a clear stress response. These results highlight the importance of sufficient recovery time if fish are to be subjected to a series of stressors during one or more days.

A13.18 DO FISH PREFER TO ASSOCIATE WITH CONSPECIFICS WITH SIMILAR METABOLIC RATES?

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Shoaling fish are known to associate with individuals of a similar phenotype to themselves to decrease the risk of predation by reducing individual conspicuousness within the group. Thus, the mechanism behind how fish assort into shoals can have implications on group dynamics as well as survivability. Previous studies have investigated fish preferences to associate with individuals of similar body size, shape and colouration, however, little is known regarding the usage of other important traits as a determinant for shoal assortment. In this study we used intermittent-closed respirometry to measure the standard metabolic rate (SMR) of 180 individual wild caught European minnows (*Phoxinus phoxinus*) to investigate if individual metabolic phenotype influences the shoal choice. Individual fish with the highest or lowest SMR were identified and given a choice to associate with size-matched groups of four fish of similar phenotypes to themselves, i.e. with either high or low SMR. It was hypothesised that fish would prefer to assort with groups of low SMR to reduce competition, however our results suggest the opposite; focal fish with both low and high SMR tend to associate with high-SMR conspecifics. This may indicate that, rather than looking to reduce competition, fish show a preference for conspecifics that have a higher metabolic rate compared to themselves.

A13.23 HISTONE (DE)ACETYLATION MODULATES EXERCISE-INDUCED SKELETAL MUSCLE PLASTICITY IN ZEBRAFISH (*DANIO RERIO*)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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Aerobic exercise has a positive impact on health and Darwinian fitness by enhancing skeletal muscle function and locomotor performance. Response of skeletal muscle to exercise involves changes in energy metabolism, calcium handling, and the composition of contractile protein isoforms, which together influence contractile properties. Histone (de)acetylation can cause short-term changes in gene expression, and may thereby mediate plasticity in contractile properties of muscle in response to exercise. The aim of this project was to determine (in zebrafish, *Danio rerio*) the traits that mediate inter-individual differences in sustained and sprint performance, and to determine whether inhibiting histone deacetylases (HDACs) mediates exercise-induced changes in these traits. High sustained performers had greater aerobic metabolic capacity (citrate synthase [CS] activity), calcium handling capacity (sarco/endoplasmic reticulum ATPase [SERCA] activity), and slow contractile protein concentration (slow myosin heavy chain [MyHC]) compared to low performers. High sprint performers had lower CS activity and slow MyHC concentration than low performers, but there were no differences in lactate dehydrogenase activity or fast MyHC concentrations. Four weeks of exercise training increased sustained performance, CS activity, SERCA activity, and slow MyHC concentration. Inhibiting HDACs increased slow MyHC concentration in untrained fish but not in trained fish. However, inhibiting HDACs reduced SERCA activity, which was paralleled by a reduction in sustained and sprint performance. Understanding how HDACs mediate skeletal muscle plasticity and locomotor performance is likely to have future medical and ecological significance.

A13.24 STRESS RESPONSE AND RECOVERY IN BANK VOLES FROM A MULTIDIRECTIONAL SELECTION EXPERIMENT

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The scope of response to a stressor, as well as a resistance to stress, are reflected in the activity of Hypothalamo-Pituitary-Adrenal (HPA) axis and dynamics of its main effector hormones (glucocorticoids) levels in blood. Modulation of the HPA axis activity may aid adaptation to different selection pressures. This problem can be approached using an artificial selection experiment. We asked whether the HPA axis sensitivity differs between lines of bank voles (*Myodes glareolus*) selected in three directions: high aerobic exercise metabolism (A), predatory behavior (P), ability to cope with low-quality herbivorous diet (H), and unselected control lines (C). We compared baseline plasma corticosterone (CORT) level and its changes in response to restraint stress or to maximum pharmacological stimulation. Preliminary analyses, based on partial results, did not indicate any statistically significant differences in baseline CORT between selection directions. On average, restraint stress elicited a 5.7-fold, and maximum stimulation a 8.5-fold increase in CORT concentrations, with no significant differences between selection directions. In the P lines, peak CORT resulting from the artificial stimulation lasted longer than in other lines. However, the post-peak recovery of CORT to the base level tended to be faster in animals from both P and A lines than in those from the C and H lines. This suggests that selection for traits requiring either burst high energy output or alertness to unexpected triggers in mildly stressful conditions resulted primarily in an improved ability of recovering after exposure to short periods of stressful conditions. (Funding: NCN 2014/13/B/NZ8/04683 and UJ:DS/WBINOZ/INOS/757)

A13.25 DIFFERENTIAL RESPONSE OF ION TRANSPORTERS TO NITRIC OXIDE IN STRESSED FISH BRAIN

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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The neuronal circuitries of brain and its ion transporters contribute to the physiological homeostasis in fishes. The sensitivity of these physiological machineries to the environmental challenges further implies its plasticity and that contributes to stress adaptation. As transporters that provide the driving force for many other transport systems, Na^+/K^+ -ATPase, H^+/K^+ -ATPase and H^+ -ATPase are vital for Na^+/K^+ , and H^+ homeostasis in fish brain. Nitric oxide (NO), a gas transmitter, is known for its role in ion transport in the osmoregulatory tissues of fish. However, the mechanism of NO action on ion transporter function has not yet understood in stressed fish brain. Subsequently, we investigated the response pattern of ion transporters to the activator or inhibitor of NO in the brain of hypoxic or net-confined fish. We found that changes in the availability of NO can alter the functions of these transporters including the mRNA expression of nka alpha subunit isoforms and NKA protein abundance. We also found that these transporters could regain its basal activity level during recovery or ease response. These results indicate that the ion transporters have differential sensitivity to NO and are able to mitigate the stress-induced disturbance in ion transporter functions in fish brain (Supported by a grant from DBT, New Delhi and Emeritus Scientist scheme of KSCSTE).

A13.26 TIME COURSE AND MECHANISM OF PHENOTYPIC FLEXIBILITY OF INTESTINAL ENZYMES DURING ONTOGENY IN HOUSE SPARROW

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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House sparrows during ontogeny are challenged to adjust digestive capacity to changes in food composition. Parents feed nestlings after hatch with a protein-(insects)-based diet and then within a timeframe of 12-days. We previously demonstrated that nestlings have small spare-digestive capacity (<20%) and that they mainly rely on phenotypic flexibility of intestinal enzyme activities [e.g., sucrase (SI), maltase (MG) and aminopeptidase-N (APN)] when exposed to an increase/decrease in the respective substrate in the diet. However, our understanding of the molecular mechanisms underlying these activity changes and its time-course are unknown. Therefore, 3-day-old nestlings were captured and fed either a low carbohydrate-high protein diet or a high carbohydrate-low protein diet. Then birds were divided in groups: (A) kept on the original diet until 30-days-old, (B) at age 12-days-old were switched to the alternative diet until 30-days-old, (C) fed either diet for 3-days before being switched to the other diet. Intestine was harvested in groups (A) and (B) at 3, 6, 12 and 30-days-old, and (C) 24, 48, and 72-hours after diet switch. Samples were analyzed for levels of activity and RNA of MG-like-enzyme (NCBI-accession# GQ919053.1) and APN. Experiment-(A), (B) and (C) showed that activities and RNA levels of enzymes varied in the direction of the main dietary substrate indicating a transcriptional-compatible mechanism of regulation underlying the phenotypic flexibility. Additionally, experiment-(C) demonstrated a (with no further changes) enzyme digestive response to a substrate switch in 24-h. NSF-IOS-1354893, CONICET-PIP-834.

A13.27 SLEEP DEVELOPMENT AND SOUND RECOGNITION IN KING PENGUINS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

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King penguins are exposed to incessant noise throughout their life on land. King penguin chicks and juveniles do not go out to sea until they have fully lost all their chick (brown coloured) plumage after 10-11 months. During this time, maturing chicks undergo various stages of development. This might affect their sleeping behaviour as brain development requires more rest time. We hypothesise that sleeping chicks respond to sound stimuli only at significantly higher volumes than do juveniles and adults. Furthermore, we expect chicks to move away from the sound less often. Chicks without parents stay in crèches, seeking refuge in numbers to protect them from land predators. We randomly applied 1 of 6 sound stimuli (each 15 s in duration, during which the volume steadily increased) to both wakeful and sleeping penguins at three life stages: (1) chick, (2) juvenile, (3) Adult. We recorded the penguins' response and quantified their response time, walk time (time at which they walked away from the sound source), and response rate. Overall we found that chicks and adults show an increased response time when sleeping compared to when awake, while juveniles did not. In terms of time until walking away from the stimulus, chicks and juveniles take longer to respond than adults. This may be because younger penguins are more cautious about leaving the area. Interestingly, sleeping chicks respond up to 5 s later to the playback of an orca sound than do juveniles or adults. Further results will be discussed in the presentation.