

**ABSTRACT BOOK**

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# ANIMAL BIOLOGY ABSTRACTS

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# A1 DEVELOPMENTAL BIOMECHANICS OF MOTOR SKILLS

ORGANISED BY: PROF JOHAN VAN LEEUWEN (WAGENINGEN UNIVERSITY, NETHERLANDS) & PROF PETER AERTS (UNIVERSITY OF ANTWERP, BELGIUM)

## A1.1 ACQUIRED VERSUS INNATE PREY CAPTURING SKILLS IN SUPER-PRECOCIAL LIVE-BEARING FISH

📅 TUESDAY 5 JULY, 2016 ⌚ 13:45

👤 MARTIN LANKHEET (WAGENINGEN UNIVERSITY, NETHERLANDS)

Live-bearing fish start hunting for mobile prey within hours after birth, an example of extreme precocity. Because prenatal, in utero, development of this behaviour is constrained by the lack of free-swimming sensory-motor interactions, immediate success after birth depends on innate, evolutionary acquired patterns. Optimal performance however requires flexible adjustment to an unpredictable environment. To distinguish innate from postnatally acquired patterns we analyzed over 2000 prey capture events for 28 Metallic livebearers (*Girardinus metallicus*; Poeciliidae), during their first three days after birth. We show that the use of synchronous pectoral fin beats for final acceleration and ingestion is truly innate. It allows for direct control while avoiding head yaw, supporting immediate success. Rapid development of eye movements and body curvatures, however, show that eye-tail coordination requires postnatal learning and calibration. The combination of innate motor programs and rapid, postnatal development reveal how super-precocial animals optimize survival into adulthood.

## A1.2 BENDING MOMENT DYNAMICS DURING SWIMMING OF DEVELOPING ZEBRAFISH LARVAE

📅 TUESDAY 5 JULY, 2016 ⌚ 14:15

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Zebrafish larvae are able to swim immediately after hatching, making effective escape manoeuvres at two days post fertilization (dpf). From 2 to 5 dpf, larval zebrafish improve swimming performance by increasing their tail-beat frequency and amplitude (Van Leeuwen et al. (2015) J.R. Soc. Interface 12:20150479). During these first days of development, the larvae's muscle system changes rapidly, while it continues functioning to power swimming. This requires them to use their muscles differently across development. A first step towards understanding how the larvae achieve this and how they change their performance, is by computing the time-dependent internal bending moment distributions along the body during swimming. This allows us to assess the changes

in local bending power as the fish grows. We developed a combined experimental and computational approach for reconstructing time-resolved bending moment distributions from high-speed videos of free-swimming larvae (2-12 dpf). First, we reconstruct the three-dimensional position, orientation and body curvature from these images. We feed these reconstructions into a computational fluid-dynamics solver in order to calculate the flow field and the fluid forces along the fish's body. Finally, we combine the motion of the longitudinal body axis and the external fluid forces as input for an optimization procedure to calculate the best fitting time-dependent bending moment distribution. The dynamics of these bending moments provide novel insight in the developmental mechanics of swimming across the first stages of zebrafish.

## A1.3 HOW TAIL-BEAT FREQUENCY AND BODY CURVATURE AFFECT SWIMMING PERFORMANCE IN LARVAL ZEBRAFISH

📅 TUESDAY 5 JULY, 2016 ⌚ 14:30

👤 GEN LI (CHIBA UNIVERSITY, JAPAN), ULRIKE K MÜLLER (CALIFORNIA STATE UNIVERSITY FRESNO, UNITED STATES), HAO LIU (CHIBA UNIVERSITY, JAPAN), JOHAN L VAN LEEUWEN (WAGENINGEN UNIVERSITY, NETHERLANDS)

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Small undulatory swimmers such as larval zebrafish operate in the intermediate Reynolds regime and experience relatively high drag during cyclic swimming. Experimental observations (J.R. Soc. Interface 12:20150479) demonstrated (a) that larval zebrafish tend to increase both tail-beat frequency and amplitude with swimming speed and (b) a negative power relationship between Strouhal number and Reynolds number during cyclic swimming.

To elucidate the underlying mechanisms, we developed an integrated 3D computational approach of hydrodynamics and free-swimming body dynamics that couples the Navier-Stokes (NS) equations to the equations of undulating body motion. A numerical approach is required to analyze the highly non-linear nature of the dynamics of large-amplitude undulatory swimming in the intermediate Reynolds regime. Using the model, we explored how tail-beat frequency and amplitude of lateral curvature along the body affect swimming performance (in terms of speed, fluid dynamic efficiency and cost of transport). The explored parameter space extends beyond the experimentally observed frequency-amplitude combinations in larval zebrafish.

Our computations predict that increasing both frequency and amplitude to swim faster improves swimming performance, which agrees with previous experimental observations. This suggests that fish larvae adjust their body kinematics to optimize swimming performance. In addition, a robust negative power relationship between  $Re$  and  $St$  was predicted, again in line with experimental

observations, and irrespective of the employed combinations of frequency and curvature amplitude. The coupling between  $R_e$  and  $S_t$  is not an effect of kinematic optimization, but results from fluid dynamic constraints.

## A1.4 FIRST STEPS – THE EMERGENCE OF WALKING IN CHILDREN

TUESDAY 5 JULY, 2016 14:45

NADIA DOMINICI (VU UNIVERSITY, NETHERLANDS)

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When neonates are supported for ~70-80% of their weight, they instinctively 'walk' as their feet come into contact with a horizontal surface. This 'stepping reflex' is hardwired in our neural circuitry. In normally developing children, however, the ability to walk independently emerges only about one year later.

Walking involves the coordinated activation of numerous muscles to provide forward progression while maintaining balance. In this talk I will show that the central nervous system reduces the complexity of muscle coordination to a small number of elementary commands. Like the phonetic units used in speech, these elementary locomotor commands, or locomotor primitives, can be combined in a flexible manner to generate a rich behavioural repertoire, including walking and running at different speeds, walking forwards or backwards, or walking with variable loads.

I will discuss how the number and type of locomotor primitives change with development in animals and humans. I will focus in particular on the role of balance and body-weight control when independent locomotion emerges in toddlers.

## A1.5 FROG LOCOMOTION. HOW TO DEVELOP TWICE: ONCE FOR WATER; THEN FOR LAND

TUESDAY 5 JULY, 2016 16:00

ROB S JAMES (COVENTRY UNIVERSITY, UNITED KINGDOM)

Most frogs undergo development for a tadpole phase in water, followed by further development for an adult phase on land. The tadpole initially swims via waves of neuromuscular activity travelling down the tail. Most organs in the body are remodelled, including resorption of the tail and the growth of legs, ready for the emergence onto land. Gradually the hind legs begin to produce useful power, to assist the largely tail based locomotion, until the metamorph can use tail or limb or combined tail and limb powered locomotion in water. The adult frog uses its legs to power jumping on land and swimming in water. Such alterations in locomotor performance require coordinated changes in anatomy and neuromuscular physiology. Environmental cues, such as the presence of predators, can modulate the morphological changes undertaken, altering rates of development and the shape of the animal, in turn affecting locomotor performance and likely predation risk.

## A1.6 MOTOR SKILL DEVELOPMENT AND OPTIMAL MOVEMENT SPEEDS IN PREDATOR-PREY INTERACTIONS

TUESDAY 5 JULY, 2016 16:40

ROBBIE S WILSON (UNIVERSITY OF QUEENSLAND, AUSTRALIA)

How fast should animals move when trying to escape predators or capture prey? Most studies of animal performance assume faster is always better but this ignores the costs of high-speed movements on the ability to successfully perform motor tasks. Because motor control declines as animals move faster, an animal's movement speed should reflect a balance between the benefits of moving fast against the costs of decreases in motor control and manoeuvrability. Using a medium-sized semi-arboreal marsupial, the Australian northern quoll (*Dasyurus hallucatus*), I explore the costs of high movement speeds on the accurate placement of their feet (motor control) when moving on substrates differing in task difficulty (varying beam widths). By developing a model of optimal movement speeds for prey when attempting to escape predators, I then test whether the movement decisions of northern quolls reflect the underlying trade-off between speed and motor control, the costs of mistakes, and the ability to improve motor control with practice and throughout development.

## A1.7 PRIMATE INFANCY AND THE MECHANICS OF QUADRUPEDAL DEVELOPMENT

📅 TUESDAY 5 JULY, 2016 ⌚ 17:10

👤 FRANÇOIS DRUELLE (LABORATORY FOR FUNCTIONAL MORPHOLOGY BIOLOGY DEPARTMENT UNIVERSITY OF ANTWERP, BELGIUM), GILLES BERILLON (UMR 7194 CNRS-MNHN, FRANCE), PETER AERTS (LABORATORY FOR FUNCTIONAL MORPHOLOGY BIOLOGY DEPARTMENT UNIVERSITY OF ANTWERP, BELGIUM)

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Looking at a newborn primate, one notices its inability to move by itself and therefore its strict dependence to the mother. Soon, however, the young primate is able to perform independently. Between these two stages of early life, (loco) motor control develops gradually. On the one hand, the control of body movements seems to be mediated by neuro-motor maturation. On the other hand, because limbs and body are subjected to dramatic changes in shape and size, these too likely have important impact on locomotor performance. This raises the following question: how do the developmental changes of the interlimb coordination of quadrupedal walking relate to the intrinsic morphological (size, shape) and dynamical (inertia) properties of the limbs and body? At the Primatology Station of the CNRS we were able to study six infant baboons at two instants in their development, i.e., when they just begin foraging independently and when they are autonomous. We found that fore- and hindlimbs, at the level of the convergence of the natural pendular period, have a significant and positive impact on the interlimb coordination pattern, thus probably facilitating, very early in development, the control of the coordination. Nevertheless, because an improved control of the interlimb coordination points at a proceeding neuromotor maturation, the importance of neuromotor control relative to the intrinsic morpho-dynamics of the limbs, appears to increase gradually during infancy.

## A1.8 WALK THE LINE - GAIT DEVELOPMENT IN PIGLETS

📅 TUESDAY 5 JULY, 2016 POSTER SESSION

👤 PETER AERTS (UNIVERSITEIT ANTWERPEN, BELGIUM), CHARLOTTE VANDEN HOLE (UNIVERSITEIT ANTWERPEN, BELGIUM), JANA GOYENS (UNIVERSITEIT ANTWERPEN, BELGIUM), SARA PRIMS (UNIVERSITEIT ANTWERPEN, BELGIUM), CHRIS VAN GINNEKEN (UNIVERSITEIT ANTWERPEN, BELGIUM)

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In pig farming, genetic selection and the use of different management techniques has led to increasing litter sizes. These litters are generally characterized by a heterogeneous birth weight and a higher mortality. Recently, it's been shown that increasing piglet vitality could be key to boosting both profitability and welfare. In order to evaluate the effect of certain interventions (e.g. supplementation) on piglet vitality, there is a need for an unbiased assessment of vitality. Studies propose locomotion as an important paradigm for piglet vitality. However, a baseline of the development of locomotion and associated gait characteristics is lacking for piglets. To this end, spatio-temporal gait characteristics (e.g. stride and step lengths, stride frequencies and duty factors) of normal piglets (normal birth weight and vitality) were analyzed to study inter-limb coordination. Videorecordings and associated digitalization of the footfalls were made of piglets walking through a corridor at several time points (< 96 hours). The parameters reached stable values between 26 and 28 hours after birth, with a symmetry indices hovering around 0%, suggestive for a matured locomotion pattern with great contra-lateral symmetry. Often, parameters evolved to showing similar values for all legs (e.g. stride length, stride frequency and stance duration) or showed a clear difference between front and hind (e.g. step length, swing duration and duty factor).

# A2 HOW DOES ENERGY CONSTRAIN ECOLOGY?

**ORGANISED BY:** DR LEWIS HALSEY (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM), DR MIKE SCANTLEBURY (QUEEN'S UNIVERSITY BELFAST, UNITED KINGDOM) & PROF TERRIE WILLIAMS (UNIVERSITY OF CALIFORNIA, SANTA CRUZ, UNITED STATES)

**SESSION SPONSORED BY:** AMERICAN PHYSIOLOGICAL SOCIETY (APS)

## A2.1 MOST ANIMALS DIE BEFORE REPRODUCING: CONSIDERING THE ROLE OF ENERGETICS IN JUVENILE MORTALITY

📅 THURSDAY 7 JULY, 2016 ⌚ 08:55

👤 LEWIS HALSEY (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM)

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The ultimate goal of animals is to maximise their reproductive fitness, and so their strategy should centre on maximising the energy that they expend on reproduction over their lifetime. The perceived wisdom has been that the limit to the rate animals can assimilate energy is the relative scarcity of food in their environment, which of course will be affected by their capacity to forage for it. However, relatively recent theories purport that for endotherms, available energy is often intrinsically limited, typically because energy utilisation generates heat which if unchecked leads to hyperthermia (Speakman and Krol, 2010, JAE 79:726). Theories around energy allocation decisions and optimal foraging, and the empirical data to test those theories, tend to focus on adult animals. But what about all the juvenile animals that never even make it to sexual maturity? Might they not often die of starvation? If they do, then this could be a stark example of the environment being the limiting factor to an animal's rate of energy assimilation and, most importantly, this constraint being highly deterministic of that animal's lifetime reproductive success. Put simply, most animals die young, and this is catastrophic, of course, for their reproductive fitness. Thus to understand the full role that energy plays in driving animal ecology, it is essential that we consider its relevance to the masses of young animals that perish before adulthood. My talk sets out to establish this line of enquiry as an under-explored but crucial facet of animal ecology.

## A2.2 PATTERNS OF ENERGY LOSS AND CONSEQUENT MORTALITY RISK OVER WINTER: THE ROLE OF INDIVIDUAL VARIATION IN METABOLIC AND BEHAVIOURAL FLEXIBILITY

📅 THURSDAY 7 JULY, 2016 ⌚ 09:12

👤 NEIL B METCALFE (UNIVERSITY OF GLASGOW, UNITED KINGDOM), KARINE SALIN (UNIVERSITY OF GLASGOW, UNITED KINGDOM), GRAEME J ANDERSON (UNIVERSITY OF GLASGOW, UNITED KINGDOM), SONYA K AUER (UNIVERSITY OF GLASGOW, UNITED KINGDOM)

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Retention of energy stores is essential for the overwinter survival of many temperate and polar animals, yet there is often marked variation within a species in the rate at which these stores are depleted, with consequences for direct and indirect risks of mortality (e.g. through starvation and/or greater exposure to predators while foraging). We hypothesised that intraspecific variation in rates of overwinter energy depletion could be explained by differences in metabolic and/or behavioural flexibility in response to food scarcity, and tested this idea using overwintering brown trout (*Salmo trutta*). Decreasing food availability over winter led to a decline in lipid stores across all fish and at a rate that was comparable to that observed in wild overwintering fish. It also led on average to reductions in both metabolic and activity rates, but there were striking differences among fish in their responses. This variation was directly related to individual differences in the rate of lipid depletion: the smallest lipid depletion occurred in those individuals that had the greatest reductions in metabolism and/or activity. However, changes in metabolism and in activity were negatively correlated: fish that decreased their SMR to a greater extent tended to increase their activity rates, and vice versa. Physiological causes and ecological consequences of this intraspecific variation in energetic and behavioural strategies for coping with seasonal food scarcity will be discussed.

## A2.3 DO ENERGETICS DRIVE THE LINK BETWEEN BOTFLY PARASITISM AND OVERWINTER SURVIVAL IN CHIPMUNKS?

THURSDAY 7 JULY, 2016 09:25

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Eastern chipmunks (*Tamias striatus*) are food-storing hibernators distributed throughout the deciduous forests of eastern North America. Chipmunks hoard food in an underground burrow where they survive winter using torpor. Chipmunks are commonly infected by larval botflies (*Cuterebra emasculator*), resulting in a rather grotesque infection. The larvae develops in a subcutaneous capsule on the abdominal region of the chipmunk, breathing and excreting through a skin pore until it reaches maturity (~1g). Botfly parasitism has a detrimental effect on chipmunk overwinter survival, and here I discuss the possibility that this effect is driven by energetics. From an energetics point of view, botfly parasitism is doubly penalising for chipmunks because it simultaneously increases maintenance costs (resting metabolic rate; RMR) and reduces thermogenic capacity (cold-induced  $VO_{2max}$ ). Consequently, there is a negative correlation between the number of botfly parasites and factorial aerobic scope (FAS; ratio of  $VO_{2max}$  over RMR). When looking across species of rodents, there is a strong negative relationship between FAS and environmental temperature in heterothermic but not in homoeothermic rodents. This suggests that using torpor in cold environments requires a combination of low maintenance costs and high thermogenic capacity. Thus, energetic constraints may explain the detrimental effect of botfly parasitism on chipmunk survival, because chipmunks need a high thermogenic capacity to warm-up from torpor, yet they also need low maintenance costs to save energy and survive winter on a fixed food supply.

## A2.4 LINKING PARASITISM AND LIFE-HISTORY: NOVEL QUESTIONS WITH A NOVEL ENERGETIC APPROACH

THURSDAY 7 JULY, 2016 09:50

OLIVIA HICKS (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM), SARAH BURTHE (CENTRE FOR ECOLOGY AND HYDROLOGY, UNITED KINGDOM), FRANCIS DAUNT (CENTRE FOR ECOLOGY AND HYDROLOGY, UNITED KINGDOM), MOTOHIRO ITO (THE UNIVERSITY OF TOKYO, JAPAN), JONATHAN GREEN (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM)

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Many species exhibit reproductive skew with some individuals consistently more successful than others. The mechanisms underpinning this skew are often poorly understood yet can help us understand how individual variation can have consequences for population level changes. Recent work has illustrated that parasitism may play a crucial role in driving reproductive skew but may have differential impacts in different environmental conditions. We suggest that our understanding of how parasitism interacts with both intrinsic drivers and environmental conditions to determine breeding performance can be greatly improved by considering energetics, since many life-history processes can be

quantified through their impacts on rates of energy use and gain. Using a novel endoscope technique to quantify parasite load and bio-logging devices to estimate behaviour-specific energy expenditure in European shags we are able to determine the energetic cost of parasitism and understand how individual responses may vary with changing environmental conditions. Here we present analyses on a population of European shags that suggest that the cost of different behaviours varies with parasite load, as does the amount of time allocated to them. This work provides a potential mechanism linking the energetic cost of parasitism to its role in driving reproductive skew.

## A2.5 ENERGY CONSTRAINTS ON DISPERSAL AND RANGE EXPANSION AFFECT GEOGRAPHICAL PATTERNS OF SPECIES DIVERSITY

THURSDAY 7 JULY, 2016 10:02

DOUGLAS S GLAZIER (JUNIATA COLLEGE, UNITED STATES)

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A major goal of macroecology is to provide individual-based mechanistic explanations for broad ecological patterns, including regional variation in species diversity. Often these explanations focus on energy use because of its fundamental importance in supporting all biological activities. In this presentation I discuss two hypothetical, unexplored ways by which organismal energetic constraints on dispersal and range expansion may affect geographical variation in species diversity. The first mechanism is illustrated by a comparison of patterns of geographical population differentiation and species diversity among mammal taxa with different modes of locomotion. Energy costs of locomotion per distance travelled are very high in subterranean species, intermediate in terrestrial species, and lowest in flying species. Correlational evidence supports the hypothesis that high locomotor costs inhibit dispersal and gene flow, thus increasing geographic population differentiation and speciation. The second mechanism is illustrated using an analysis of latitudinal diversity gradients (LDGs) exhibited by four major taxa of marine phytoplankton, carried out with my colleague Matt Powell. These taxa were chosen because of their excellent fossil record and because two taxa with calcareous shells show the common trend of increasing species richness toward the tropics, whereas the other two with siliceous shells exhibit opposite trends. Our analysis shows that asymmetric range expansion (ARE), rather than differential speciation and extinction rates, has caused both types of LDGs. We further hypothesize that taxic differences in ARE (and thus LDGs) relate to differences in how temperature affects the energetic costs of maintaining mineralized shells in calcareous versus siliceous phytoplankton.

## A2.6 VARIATIONS IN ENERGY STORAGE METABOLISM DISCRIMINATE FRESH AND BRACKISH/SALTWATER ECOTYPES IN AMERICAN GLASS EELS

THURSDAY 7 JULY, 2016 10:14

MÉLANIE GAILLARD (UNIVERSITÉ DU QUÉBEC À RIMOUSKI INSTITUT DES SCIENCES DE LA MER DE RIMOUSKI, CANADA), LOUIS BERNATCHEZ (UNIVERSITÉ LAVAL, CANADA), CÉLINE AUDET (UNIVERSITÉ DU QUÉBEC À RIMOUSKI INSTITUT DES SCIENCES DE LA MER DE RIMOUSKI, CANADA)

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Recently, different ecotypes characterized by their migration form, have been genetically documented in the American eel, *Anguilla rostrata*. The aim of this study was to verify if energy status and differential abilities in mobilizing energy reserves could discriminate freshwater and brackish/saltwater ecotypes. To do so, analyses were done on glass eels at recruitment according to location of capture, date and salinity preference using eco-physiological and molecular tools. Salinity preference did not differ between ecotypes. Instead, we observed spatial and temporal variations adding to the body of evidence of genetic and environmental controls in the differentiation of ecotypes. Compared to the brackish/saltwater ecotype, the freshwater ecotype was larger and more pigmented, had 73.8% lesser triacylglycerol content and 67.7% higher glycogen content, and overexpressed 7.65 and 3.25 times respectively the transcripts of bile salt activated and triacylglycerol lipases. No variation in transcripts of glycogen phosphorylase, leptin and ghrelin was observed between ecotypes. For both ecotypes, level of pigmentation was higher and energetic reserves were lesser in glass eels arriving two weeks later. Our results suggest the existence of differential regulation mechanisms relative to energy metabolism between ecotypes and allow us to propose a new model of the physiological mechanisms underlying the recruitment of freshwater and brackish/saltwater ecotype in American glass eel. This new biological information contributes to the building knowledge on the distribution of ecotypes and on the internal factors involved in glass eel migration regulation, giving new indications to improve conservation measure for this species declared 'threatened' in Canada.

## A2.7 ONE FLAP AT A TIME: AN IN-SITU STUDY LINKING INSTANTANEOUS FLIGHT BEHAVIOUR TO FORAGING TRIP MOVEMENTS IN KITTIWAKES

THURSDAY 7 JULY, 2016 10:55

PHILIP M COLLINS (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM), JONATHAN A GREEN (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM), KYLE ELLIOTT (MCGILL UNIVERSITY, CANADA), PETER J. A. SHAW (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM), LEWIS G HALSEY (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM)

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Flying over the ocean to find patches of food is energetically expensive for seabirds, as such they must fly efficiently to ensure

that energy expended does not overwhelm energy gained while foraging. With such a sensitive energy budget, fine details about where, when and how a seabird expends its energy in the pursuit of food could determine its fate; whether it reproduces, indeed whether it survives. With GPS trackers we have successfully determined where seabirds forage, yet the finer details regarding flight behaviour during foraging trips have remained less well studied. Now, however, by coupling GPS devices with accelerometers we can study in-situ flight behaviour at a sub-second level of detail. By combining these datasets we can measure flight parameters while accounting for environmental conditions, thus allowing us to start unravelling the energetic relationship between instantaneous body movement and observed flight patterns.

Using a unique dataset comprising of combined accelerometry and GPS data from 47 incubating kittiwakes on Middleton Island, US we have calculated a range of flight parameters indicative of individual flight effort. By coupling data from these devices with weather data we have accounted for wind speed and direction in order to identify how individuals adjust their behaviours in response to different conditions. Preliminary analysis has revealed variation in fine-scale flight characteristics within and between individuals as well as general trends found in flight behaviour under different wind conditions. The energetic implications of flight characteristics linked to foraging trip patterns will be further examined and presented.

## A2.8 TO FLAP, OR NOT TO FLAP: THAT IS THE QUESTION

THURSDAY 7 JULY, 2016 11:07

CHARLES M BISHOP (BANGOR UNIVERSITY, UNITED KINGDOM)

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During locomotion, animals may modulated their behaviour as a result of state-dependent conditions or due to the impact of environmental factors that influence the energetic costs. During forward flight, birds have the option of operating between the two extremes of gliding with a fixed-wing position or undergoing fast powered flight with rapid wing flapping. During long foraging flights or while on migration, energy utilisation must be allocated with consideration of both the short term and long-term potential benefits. Measures of heart rate and dynamic body acceleration can be used to provide insight into the optimal strategy for flight. Bar-headed geese (*Anser indicus*) on migration never stopped flapping their wings although they greatly modulated the power of each flap within a relatively narrow band of wingbeat frequency. Manx shearwater (*Puffinus puffinus*), alternate between pure gliding and a highly constrained power per flap, such that the number of flaps in a period of flap-gliding modulates power output. Pigeons (*Columba livia*) modulate both power per flap and wingbeat frequency. These different flight styles will be discussed within the context of rates of energy consumption and optimal flight performance.

## A2.9 AN OVER-COST OF BEING A PELAGIC BIRD: A POSSIBLE ENERGETIC CONFLICT BETWEEN THERMOREGULATION AND DIGESTIVE PROCESSES

THURSDAY 7 JULY, 2016 11:27

AGNÈS LEWDEN (IPHC-CNRS, FRANCE), TESSA VAN WALSUM (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM), BATSHÉVA BONNET (CEBC-CHIZÉ, FRANCE), CAROLINE BOST (CEBC-CHIZÉ, FRANCE), YVES HANDRICH (IPHC-CNRS, FRANCE)

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The energetic cost of foraging activities in King Penguin consists to reach favourable areas, realises depth diving to attempt fish patch and resting in high latitude cold water. Several studies have shown that resting in cold water could be represent a more expensive cost than realized depth diving. Indeed, this paradox is probably linked with contrasting thermoregulation processes. During daylight, a general hypothermia occurs and is believed to reduce energy expenditure. At sunrise occurs a re-warming to normothermia, contributing to increase heat-loss during the night. We hypothesise an energetic conflict between thermoregulation and digestive processes. During daylight, the organism may be unable to assimilate the end product of prey digestion (free fatty acids) inside the peripheral subcutaneous adipose tissues (SAT), because skin is no more blood perfused. During the night, re-warming and re-connecting to blood circulation peripheral tissues could be inevitable to end the assimilation of FFA inside the SAT. In a first step, we have reproduced the conditions of a resting night at sea and events of rewarming skin temperature, using a water tank in which king penguins equipped with four internal temperature tags were maintained several days. In a second step, we have tested a generalisation of our hypothesis studying body temperature variations on penguins fast and feed. Finally we have measured the cost to maintain normothermia in cold water sometimes during all night long.

## A2.10 PHENOTYPIC RESPONSES TO HIGH ALTITUDE

THURSDAY 7 JULY, 2016 13:50

KIM HAMMOND (UNIVERSITY OF CALIFORNIA RIVERSIDE, UNITED STATES)

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For any animal living in highly variable or extreme habitats we expect to find adaptations that permit life. Over the past decade we are learning to distinguish between genetically hard wired components, those that are more physiologically plastic and, more recently, those components that are transcriptionally up-regulated or epigenetic. The result of this broad-spectrum research is that we are finding that the physiology life in extreme habitats is far more complex and dynamic than we thought. I will talk about several examples of this type of work but I focus mainly on Deer Mice (*Peromyscus maniculatus*) that live in both high and low altitudes across North America. These mice possess genetic adaptations to

life at high altitude but my research has shown that they also are capable of phenotypic increases in the capacity of intake tissues (gut and respiratory surfaces) in the face of increasing demands for both energy and oxygen. Other studies show that deer mice in the same species show increases in the oxygen diffusion capacity of exercising muscles under hypoxic conditions. If energy expenditures are to be maintained in the face of high demands both the ability to acquire oxygen and the fuel (glucose) for that respiration must be increased. Although deer mice represent a model system with remarkable plasticity and flexibility they also are illustrative of the fact that many different types of organisms may have far more (or less) capacity to acclimate and adapt to changes in the environment than we previously knew.

## A2.11 BRAVING THE COLD: ALTITUDE ANCESTRY AND DEVELOPMENTAL PLASTICITY OF THERMOGENIC CAPACITY IN THE NORTH AMERICAN DEER MOUSE, *PEROMYSCUS MANICULATUS*

THURSDAY 7 JULY, 2016 14:15

CAYLEIGH E ROBERTSON (MCMASTER UNIVERSITY, CANADA), GRANT B MCCLELLAND (MCMASTER UNIVERSITY, CANADA)

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The combination of low oxygen and temperature at high altitude is particularly challenging, especially for small mammals. However, despite the high energetic demands of heat production, high altitude native deer mice have an enhanced thermogenic capacity compared to low altitude congeners. Part of this difference in performance may involve early life environmental experience during development of high altitude natives. To understand the influence of developmental plasticity we used deer mice native to low (LA; 400 m a.s.l.) and high altitude (HA; 3500 m a.s.l.) born and raised in common lab conditions. Early life exposure to cold (14°C) was introduced pre- or post-natally (0-30 days). We tested the hypothesis that early exposure to low temperatures would shift developmental trajectories of thermo-effector organs, permanently enhancing adult thermogenic capacity. We determined the onset of thermogenesis in response to acute cold in pups aged 6-10 days using indirect calorimetry. This was combined with measures of pup growth rates and the maturation of the primary thermoeffector organs, skeletal muscle and brown adipose tissue (BAT). Finally, we determined maximum thermogenic capacity of cold-reared mice as adults. Postnatally cold-exposed LA pups were able to metabolically respond to an acute cold challenge 4 days earlier than controls. This coincided with increased BAT growth in both populations. Contrary to our predictions only LA native mice, exposed to cold postnatally, had an enhanced adult thermogenic capacity relative to warm-reared controls. These data suggest that developmental plasticity in response to rearing temperature may be lost in HA natives.

## A2.12 HIGH-ALTITUDE ANCESTRY ALTERS THE PLASTICITY OF MUSCLE MITOCHONDRIA IN CHRONIC COLD AND HYPOXIA IN DEER MICE

THURSDAY 7 JULY, 2016 14:27

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Mitochondria are essential for aerobic energy production and limitations on ATP supply can impact whole-animal performance. Small mammals living at high altitude face the competing energetic challenge of maintaining thermogenesis in a hypoxic environment that can impair aerobic ATP supply. This raises a potential trade-off at the mitochondria, because thermogenesis would be enhanced by mitochondrial uncoupling whereas ATP supply in hypoxia is best preserved with mitochondria that have high phosphorylation efficiency for oxygen use. To examine this potential trade-off deer mice (*P. maniculatus*) native to high- and low-altitude but lab born and raised, were acclimated to: warm (25°C) normoxia; warm hypoxia (simulated altitude of 4300m); cold (5°C) normoxia; and cold + hypoxia. We measured respiration and oxygen affinity of isolated mitochondria, mitochondrial abundance, capillarity, and fibre-type distribution in locomotory muscles. P muscle mitochondrial volume densities, ATP yields per mole O<sub>2</sub>, and maximal respiration rates using both pyruvate and palmitoyl-carnitine were all higher in warm normoxic highland mice. Generally hypoxia acclimation had little effect on mitochondrial function in lowland mice, but it reduced substrate respiration rates in highland mice (possibly to help increase mitochondrial O<sub>2</sub> affinity). Cold acclimation restored pyruvate and fatty acid respiratory capacity to control levels in highland mice, which also showed an increase in mitochondrial uncoupling. Acclimation to cold+hypoxia did not change mitochondrial physiology beyond cold alone and appeared to counteract the effects of hypoxia on highland mice. Our results suggest that both highland ancestry and plasticity affect mitochondrial physiology, and likely contributes to performance at high altitudes.

## A2.13 HOW DOES MITOCHONDRIAL FUNCTIONING CONSTRAIN ENERGY EFFICIENCY?

THURSDAY 7 JULY, 2016 14:39

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Although a great number of ecophysiological studies have focused on factors affecting energy acquisition and allocation, surprisingly few have considered energy processes at the mitochondrial level. Energy derived from the diet becomes usable only after being oxidized and converted into adenosine triphosphate (ATP) by the mitochondria. Here we illustrate the role of intraspecific variation in mitochondrial

functioning in constraining animal energetics using evidence from an ectotherm, the brown trout *Salmo trutta*. We show that conspecifics living in the same environment displayed up to a 3-fold variation in the rate of energy dissipation through mitochondrial proton leak respiration. Those that had a greater mitochondrial leak respiration may be to partially offset this leak, as revealed by a higher whole-organism metabolic rate. These individuals also had the poorest performance at high temperatures. However, it is important to note that mitochondrial properties are not fixed but change according to conditions: fasting caused disproportionate changes in mitochondrial capacities of the liver, such that substrate oxidation increased far more than did the ATP synthesis. As a result, the ATP/O ratio (the amount of ATP produced per unit of oxygen consumed, i.e. the efficiency of ATP production) decreased in response to fasting. These illustrations, combined with examples from the literature, suggest that mitochondria can be a significant constraint in the use of energy resources and their allocation into ATP. Among-individual variation in mitochondrial functioning is therefore likely to contribute to the proximate causes of differences in animal performance.

## A2.14 INDIVIDUAL VARIATION IN METABOLIC FLEXIBILITY AND ITS EFFECTS ON GROWTH IN CHANGING ENVIRONMENTS

THURSDAY 7 JULY, 2016 14:51

SONYA K AUER (UNIVERSITY OF GLASGOW, UNITED KINGDOM), KARINE SALIN (UNIVERSITY OF GLASGOW, UNITED KINGDOM), AGATA M RUDOLF (JAGIELLONIAN UNIVERSITY, POLAND), GRAEME J ANDERSON (UNIVERSITY OF GLASGOW, UNITED KINGDOM), NEIL B METCALFE (UNIVERSITY OF GLASGOW, UNITED KINGDOM)

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Phenotypic flexibility in physiological, morphological and behavioural traits can allow organisms to cope with new and changing environments. Flexibility in standard metabolic rate (SMR) may be particularly important since SMR reflects the minimal energetic cost of living and is one of the primary traits underlying organismal performance. SMR can increase or decrease in response to food availability, but consequences of these metabolic changes for growth rates and other fitness components are not well understood. We examined individual variation in metabolic flexibility in response to changing food levels and its consequences for somatic growth in juvenile wild-origin brown trout (*Salmo trutta*). Like many other organisms, larger body size often confers an advantage in competitive interactions and survival in young brown trout, so early growth rates can have important consequences for fitness. However, food availability can exhibit marked spatial and temporal variation in the freshwater streams they inhabit, so flexibility in their metabolic rates may be critical to growth. We found that SMR increased when individuals were switched to a high food ration and decreased when they were switched to a low food regime. However, individuals differed in their metabolic flexibility with important consequences for their somatic growth; individuals that increased their SMR more in response to elevated food levels grew fastest while individuals that depressed their SMR more in response to lowered food levels fared better under those conditions. These results suggest that flexibility in standard metabolic rate is a key mechanism that allows organisms to cope with variable environments.

## A2.15 VARIATION IN MUSCLE METABOLIC PLASTICITY: ONTOGENY, ENVIRONMENT, AND ALTITUDE ANCESTRY

THURSDAY 7 JULY, 2016 15:03

GRANT B MCCLELLAND (MCMASTER UNIVERSITY, CANADA), CAYLEIGH E ROBERTSON (MCMASTER UNIVERSITY, CANADA), LEANNE ZUBOWSKI (MCMASTER UNIVERSITY, CANADA), SAJENI MAHALIGAM (MCMASTER UNIVERSITY, CANADA)

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Skeletal muscle plays essential roles in locomotion, thermogenesis, and metabolic homeostasis. For mammals at high altitude, muscle phenotype may affect the ability to effectively thermoregulate and engage in endurance locomotion in low O<sub>2</sub> and temperature. A potential trade-off exists between maximizing efficiency of O<sub>2</sub> use through carbohydrate oxidation and the capacity for sustained lipid oxidation for heat production. To explore variation in muscle phenotype we used deer mice (*P. maniculatus*) native to high altitude (HA) and low altitude (LA), but born and raised in lab conditions. We examined the development of muscle phenotype over postnatal days P0-P10 (when endothermy develops), in juveniles (P21-P27), and in adults. Adult mice were acclimated to 1) warm normoxia, 2) warm hypoxia, 3) cold, 4) cold + hypoxia to assess phenotypic plasticity. We hypothesized that in HA mice muscle aerobic phenotype develops faster, show plasticity to cold but not hypoxia, according to the special grain of their native environment. We found that over P0-P10 altitude ancestry had little effect on the ontogeny of muscle fiber-type. By P21 HA mice had greater density of aerobic fibers, capillaries, and distinct myosin isoform composition. Phenotypic plasticity in adults was affected by altitude ancestry, HA mice showed little hypoxia acclimation response in aerobic properties of muscle but significant changes in enzymes for glucose metabolism. Both populations showed a strong acclimation response to cold with increases in markers of mitochondrial abundance. These changes in muscle phenotype reflect changes in whole-animal exercise fuel use and capacity for thermogenesis.

## A2.16 TURNING OFF THE HEAT DURING LACTATION: TRANSCRIPTOME PROFILING OF BROWN FAT IN LABORATORY MICE

THURSDAY 7 JULY, 2016 15:45

ELZBIETA KROL (UNIVERSITY OF ABERDEEN, UNITED KINGDOM), ALEX DOUGLAS (UNIVERSITY OF ABERDEEN, UNITED KINGDOM), DAVINA DEROU (UNIVERSITY OF ABERDEEN, UNITED KINGDOM), JOHN R. SPEAKMAN (UNIVERSITY OF ABERDEEN, UNITED KINGDOM)

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Lactation is widely recognised to be the most energy demanding period in the life cycle of small mammals. Food consumption increases rapidly in early lactation to 2–4 times the level of non-reproductive individuals, but then reaches a plateau despite 1) the continued rise in energy requirements of the offspring and 2) typically *ad libitum* food supply provided by the experimental conditions. The nature of the physiological limits to lactation is central to understanding many aspects of animal performance,

including reproductive output, foraging behaviour and thermoregulatory capabilities. Both empirical evidence and theoretical considerations have led us to postulate that lactating females are limited in their performance by the capacity to dissipate body heat. If the heat dissipation limit (HDL) theory is correct, then increases in milk production and associated lactogenic heat would require decreases in the heat generated by competitive processes such as UCP1-dependent thermogenesis in brown adipose tissue (BAT). To test this prediction, we bred MF1 laboratory mice at room temperature (21°C), measured their milk production at peak lactation and then harvested interscapular BAT for transcriptomic profiling by RNA-seq. We focused on the sets of genes that were 1) differentially expressed between lactating and non-reproductive mice and 2) significantly correlated with milk production (ranging from 95.5 to 227.4 kJ/day). The transcriptomic alterations of BAT during lactation were consistent with highly coordinated downregulation of BAT thermogenesis, with a number of pathways modified in relation to the levels of milk production, providing strong support for the HDL theory.

## A2.17 THERMOREGULATORY VARIATION IN EUROPEAN AND AFRICAN MOLE-RATS: A CASE OF CONVERGENT EVOLUTION?

THURSDAY 7 JULY, 2016 16:05

MICHAEL SCANTLEBURY (QUEEN'S UNIVERSITY BELFAST, UNITED KINGDOM), ATTILA NÉMETH (MTA-MTM-ELTE RESEARCH GROUP FOR PALEONTOLOGY, HUNGARY), DAVID CZABÁN (DEPARTMENT OF WILDLIFE BIOLOGY AND ETHOLOGY KAPOSVÁR UNIVERSITY KAPOSVÁR, HUNGARY), NIGEL C BENNETT (UNIVERSITY OF PRETORIA, SOUTH AFRICA), GÁBOR CSORBA (HUNGARIAN NATURAL HISTORY MUSEUM BUDAPEST, HUNGARY), JÁNOS FARKAS (DEPARTMENT OF SYSTEMATIC ZOOLOGY AND ECOLOGY EÖTVÖS LORÁND UNIVERSITY H-1117 BUDAPEST, HUNGARY)

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Ecological physiology aims to understand how organisms function in and respond to their natural environment, including periods that might be stressful. Examining how individuals respond to different conditions provides an indication of how species and populations survive and how they might persist under global change. Using mole-rats as model organisms, we explored the physiological responses of different species to variation in ambient conditions. Subterranean mammals are interesting model organisms because they are adapted to specific conditions (e.g. temperature, humidity, soil structure) and their populations are vulnerable to disturbance. In southern Africa, some of the most endangered mammals are fossorial, and in Europe, populations of blind mole-rats are unique, fragmented, with some species being rediscovered after a 50-year gap. We examined heat production and heat dissipation from two distantly related groups: European mole-rats (*Spalax*) and southern African mole-rats (*Cryptomys*) in response to varying ambient temperature by measuring oxygen consumption, core body temperature and surface temperature. Both groups contained an arid and a mesic-adapted species. In both circumstances, the arid-adapted species had a higher oxygen consumption and greater thermal conductivity at lower ambient temperatures than the mesic adapted species, indicating increased insulation in the latter. However, there was greater variability in surface temperature in both of the mesic species compared to the arid species. Results indicate consistent differences

across morphologically similar but phylogenetically distinct clades of subterranean mammals and that, even within specific groups, large differences in physiological responses occur.

## A2.18 RACING FROM EXTINCTION: THE HIGH PRICE OF MOBILITY IN TERRESTRIAL AND MARINE CARNIVORES

THURSDAY 7 JULY, 2016 16:17

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One of the most energetically intensive behaviors routinely performed by mammals is locomotion. Whether patrolling territories, migrating, chasing prey, searching for mates, or avoiding anthropogenic disturbance or climate change, the act of swimming and running can have marked effects on balancing energy in an individual. Standard allometric regressions for energetic transport costs predict that these locomotor costs are vastly different for marine and terrestrial mammals. However, in the wild this is not always the case. Here we examined how aquatic or terrestrial living affects the cost of a stroke or step in a wide variety of large (>25 kg) carnivores. Using accelerometer-based ECG and GPS recorders combined with open flow respirometry we found that the energy expended for a stroke or step increased with locomotor speed. At preferred speeds total stroke costs ranged from 2.4 J.kg<sup>-1</sup> stroke<sup>-1</sup> in phocid seals to 3.9 J.kg<sup>-1</sup> stroke<sup>-1</sup> for odontocete cetaceans. Step costs were markedly higher, ranging from published values of 5.0 J.kg<sup>-1</sup> step<sup>-1</sup> for herbivores and domestic mammals to 6.0-6.7 J.kg<sup>-1</sup> step<sup>-1</sup> in cougars and polar bears. Energetic differences between terrestrial and marine mammals were reduced during high speed performance. Exponential increases in drag with swimming speed resulted in a doubling of stroke costs in cetaceans compared to modest 50% increases in step costs for many fast moving runners. Thus, prolonged, high speed chases pursuing prey or engaging in flight responses to avoid humans or animal conflicts may represent an exceptional energetic challenge for marine carnivores compared to mammals that move on land.

## A2.19 HOW THE ENERGETICS OF ENGULFMENT AND FILTRATION CONSTRAIN RORQUAL FORAGING ECOLOGY

THURSDAY 7 JULY, 2016 16:30

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Baleen whales (Mysticeti) rank among the largest animals of all-time and as consequence they exhibit a unique combination of high absolute energy requirements and low mass-specific metabolic rates. Mysticetes are obligate filter feeders and meet their energy demands by feeding in bulk on small prey (copepods, krill, forage fish) suspended in seawater. A family of baleen whales, called rorquals (Balaenopteridae) evolved a specific mode of bulk filter feeding called lunge feeding that is characterized by the intermittent engulfment of extremely large volumes of prey-laden water. Lunge feeding is a high

cost, high intake mechanism that is dependent on high-density prey patches to achieve high energetic efficiency. Because high quality prey patches are often located deep in the ocean, baleen whale energy flux is constrained by the physiological and ecological trade-offs associated with diving and feeding. Here I explore recent research that uses high-resolution movement tags to quantify the kinematics of diving and feeding across the full body size range of rorqual whales. The scaling of feeding performance and diving capacity across taxa suggest a trade-off between engulfment capacity and the ability to perform longer, deeper foraging dives. At the foraging dive scale, rorquals balance the minimization of energy expenditure with the maximization of energy intake across prey density and depth gradients. These data inform hypotheses regarding optimal foraging theory, the evolution of body size, and the physiological limits to gigantism.

## A2.20 GREAT HAMMERHEAD SHARKS SWIM ON THEIR SIDE TO REDUCE TRANSPORTS COSTS

THURSDAY 7 JULY, 2016 16:55

NICHOLAS L PAYNE (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM), GIL IOSILEVSKII (TECHNION, ISRAEL), ADAM BARNETT (JAMES COOK UNIVERSITY, AUSTRALIA), CHRIS FISCHER (OCEARCH, UNITED STATES), RACHEL T GRAHAM (MARALLIANCE, BELIZE), ADRIAN C GLEISS (MURDOCH UNIVERSITY, AUSTRALIA), YUUKI Y WATANABE (NATIONAL INSTITUTE OF POLAR RESEARCH, JAPAN)

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Animals exhibit a wide range of physiological and behavioral strategies for minimizing the energetic cost of transport. The fins of aquatic animals play key roles in efficient travel and for sharks, the function of dorsal and pectoral fins are thought to be well divided: the former assist propulsion and generate lateral hydrodynamic forces during turns; the latter generate vertical hydrodynamic forces that offset sharks' negative buoyancy. Here I show that great hammerhead sharks drastically reconfigure the function of these structures, using an exaggerated dorsal fin to generate lift by swimming rolled onto their side. Tagged wild sharks spent up to 90% of time swimming at roll angles between 50 and 75°, and hydrodynamic modelling showed that doing so reduces drag - and in turn, the cost of transport - by around 10% compared with traditional upright swimming. Rolled swimming in this species appears a unique behavioral solution to minimizing energy expenditure, and points to a significant trade-off between efficiencies of travel versus those of foraging.

## A2.21 CONTROL OF LIPID METABOLISM BY PEROXISOME PROLIFERATOR ACTIVATED RECEPTORS (PPAR) ACROSS THE ANNUAL CYCLE IN A MIGRATORY BIRD

WEDNESDAY 6 JULY, 2016 POSTER SESSION

PAUL J SCHAEFFER (MIAMI UNIVERSITY, UNITED STATES), KEELY R CORDER (MIAMI UNIVERSITY, UNITED STATES), JANICE M HUSS (BECKMAN RESEARCH INSTITUTE CITY OF HOPE, UNITED STATES)

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The annual cycle of a migrating bird involves stages of substantial fatty acid storage and periods of increased fatty acid mobilization and utilization, and thus requires a great deal of phenotypic flexibility. Specific mechanisms directing stage transitions of lipid metabolism in migrants are largely unknown. We characterized the role of the nuclear receptors, peroxisome proliferator-activated receptors (PPARs), in migratory adiposity of the Gray Catbird (*Dumetella carolinensis*). Catbirds increased adipose storage during spring and fall migration and showed increased rates of basal lipolysis during migration and tropical overwintering. Expression of the PPAR target genes involved in fat uptake and storage, FABPp and Pln3, increased during pre-migratory fattening. We found significant correlation between PPAR $\gamma$  and target gene expression in adipose but little evidence that PPAR $\alpha$  expression levels drive metabolic regulation in liver during the migratory cycle.

## A2.22 THE ROLE OF OXYGEN LIMITATIONS ON PHYSIOLOGICAL PERFORMANCE IN GROUND BEETLE *CARABUS NEMORALIS*

WEDNESDAY 6 JULY, 2016 POSTER SESSION

AGNIESZKA GUDOWSKA (INSTITUTE OF ENVIRONMENTAL SCIENCES, JAGIELLONIAN UNIVERSITY, POLAND), JAN KOZLOWSKI (INSTITUTE OF ENVIRONMENTAL SCIENCES, JAGIELLONIAN UNIVERSITY, POLAND), ULF BAUCHINGER (INSTITUTE OF ENVIRONMENTAL SCIENCES, JAGIELLONIAN UNIVERSITY, POLAND)

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Terrestrial environment typically provides oxygen at higher concentration compared to aquatic habitats, but many microhabitats exist in which terrestrial animals regularly encounter hypoxia. Such hypoxic conditions may necessitate physiological, biochemical, behavioral and morphological responses of animals in order to cope with limited oxygen availability, but may also provide benefits through physical protection from predators and reduced competition for food. Under low oxygen availability, animals may face limitations to provide sufficient energy for maintenance through aerobic metabolism at a basal level, and any higher energy requirements may amplify oxygen limitations. Here, we tested metabolic rate (MR) of the ground beetle *Carabus nemoralis* (n=26) during rest (SMR), locomotor activity (LMR) and feeding (SMR + specific dynamic action - SDA) under three oxygen

regimes: normoxia (21% O<sub>2</sub>), moderate (14% O<sub>2</sub>) and severe hypoxia (7% O<sub>2</sub>). SMR and LMR did not differ between oxygen treatments (ANCOVA, p>0.05). Nonetheless, oxygen limitation had a significant influence on feeding. In both hypoxic conditions the beetles fed less, feeding time was shorter (ANCOVA, p<0.01) and metabolic rate during feeding appeared lower (ANCOVA, p<0.01) than in normoxia. Mean MR during feeding process was about half of that during LMR in hypoxia, however did not differ in normoxia (interaction treatment\*MR category, ANCOVA, p<0.05). These findings indicate that physiological processes during oxygen limitation may be differentially constrained. Thus, hypoxic habitat may be beneficial with respect to predator avoidance or food abundance, but the limited digestive performance may offset such benefits.

## A2.23 ENERGY BUDGETS OF AN ENDANGERED LEMUR IN A CHALLENGING ENVIRONMENT

WEDNESDAY 6 JULY, 2016 POSTER SESSION

BIANCA WIST (UNIVERSITY OF HAMBURG, GERMANY), JANINA BETHGE (UNIVERSITY OF HAMBURG, GERMANY), ELEANOR STALENBERG (AUSTRALIAN NATIONAL UNIVERSITY, AUSTRALIA), KATHRIN H DAUSMANN (UNIVERSITY OF HAMBURG, GERMANY)

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Understanding physiological mechanisms such as energetic constraints of endangered species is fundamental for informing conservation strategies. This applies particularly to small folivorous mammals in seasonal environments as their low nutrition diet and relatively high costs of thermoregulation make balancing energy intake and expenditure difficult. Thus, many species use physiological energy saving strategies like daily torpor or hibernation during unfavourable seasons. This study presents the first data on energetic demands of the white-footed sportive lemur *Lepilemur leucopus*, an endangered species endemic to the driest and climatically most unpredictable parts of Madagascar. We measured resting metabolic rate using indirect calorimetry (n=14) in a field set-up, during both the wet and dry season, the latter being Malagasy winter with colder ambient temperatures and food shortage. Across all measured temperatures, lemurs displayed higher metabolic rates in the dry season than in the wet season. Accordingly, no torpor or hibernation was found and energy requirements in the dry season amounted to 88% of allometric prediction values versus 50% in the wet season. Our results suggest that *L. leucopus* adjusts metabolism to the colder dry season and may also use behavioural changes such as decreased activity or diet choices to compensate for higher demands. This flexibility might help *L. leucopus* to master the challenges caused by the variations in climatic conditions due to anthropogenically induced habitat alterations or climate change. Moreover, our data highlight the importance of studying physiological parameters, such as energy budgeting, in different seasons.

## A2.24 SHEEPISH BEHAVIOUR: ACCELERATING EXPERTISE IN LIVESTOCK PRODUCTION

WEDNESDAY 6 JULY, 2016 POSTER SESSION

CHRISTINA C MULVENNA (QUEEN'S UNIVERSITY BELFAST, UNITED KINGDOM), NIKKI MARKS (QUEEN'S UNIVERSITY BELFAST, UNITED KINGDOM), RORY WILSON (SWANSEA UNIVERSITY, UNITED KINGDOM), AARON MAULE (QUEEN'S UNIVERSITY BELFAST, UNITED KINGDOM), LEWIS HALSEY (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM), MICHAEL SCANTLEBURY (QUEEN'S UNIVERSITY BELFAST, UNITED KINGDOM)

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Livestock production is facing a new set of challenges. An ever increasing human population has resulted in a continued demand for high quality but affordable animal protein. Subsequently, the number of sheep and lambs that are slaughtered annually has increased, whereas the value of lamb/mutton has decreased by 3.61% during 2015 to 2016. Livestock producers must therefore ensure that best-practice measures are implemented to improve the production efficiency whilst high standards of animal welfare and profit margins are maintained. These targets can be addressed by determining precise behavioural profiles of individual animals. Recent developments in animal logging technology show great promise in determining animal behaviour and energy expenditure of instrumented animals. We attached tri axial accelerometers to sheep which were allowed to forage freely at pasture. Activity was recorded simultaneously using a video camera. Discrete behaviours such as resting, walking, running and grazing could be clearly differentiated using the accelerometer data, as confirmed from the video recordings. The potential automated classification of data allows for easier processing of larger datasets and the potential to generate time energy budgets for sheep, which will provide invaluable information to producers regarding how the animals use energy.

## A2.25 IS THERE A LINK BETWEEN PACE OF LIFE AND PHENOTYPIC PLASTICITY?

WEDNESDAY 6 JULY, 2016 POSTER SESSION

CLÉMENCE GOURTAY (INSTITUT FRANÇAIS DE RECHERCHE POUR L'EXPLOITATION DE LA MER, FRANCE), CÉLINE AUDET (UNIVERSITÉ DU QUÉBEC À RIMOUSKI, CANADA), DENIS CHABOT (PÊCHES ET OCÉANS CANADA, CANADA), GUY CLAIREAUX (UNIVERSITÉ DE BRETAGNE OCCIDENTALE, FRANCE), PASCAL SIROIS (UNIVERSITÉ DU QUÉBEC À CHICOUTIMI, CANADA), JOSÉ ZAMBONINO (INSTITUT FRANÇAIS DE RECHERCHE POUR L'EXPLOITATION DE LA MER, FRANCE)

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Life-history studies provide a global framework for the comparison of fish responses and trade-offs when faced with ecological and environmental constraints. The pace-of-life syndrome hypothesis specifies that closely related species experiencing different ecological conditions should differ in a suite of metabolic, hormonal and immunity traits that have coevolved with the life-history particularities related to these conditions. A given set of ecological conditions favors a particular life-history strategy and could affect a whole series of traits. The idea of the pace of life syndrome finds its roots in the classic concept of *r* and *K*-selection. It also extends into the more recent concept of fast-slow life-history continuum by expanding the examination of life-history differences among species to include physiological traits. The European bass (*Dicentrarchus labrax*) and North American striped bass (*Morone saxatilis*) share common physiological and ecological features as they evolved from a common ancestor. However, these two species tend to have a contrasted life strategy. *D. labrax* has a three times shorter generation time, is sexually mature sooner, has a higher fecundity and a longevity twice shorter than *M. saxatilis*. This faster pace of life suggests that *D. labrax* is an *r*-species while *M. saxatilis* is rather a *K*-species, having a slower pace of life. In this context, the aim of this study is to compare the phenotypic response of *D. labrax* and *M. saxatilis* in order to examine the link between pace of life and plasticity and the underlying trade-offs.

# A3 COMPARATIVE CARDIO-RESPIRATORY PHYSIOLOGY

ORGANISED BY: DR MICHAEL BERENBRINK (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM) & DR GINA GALLI (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

## A3.1 IS CARDIOVASCULAR SCOPE IN ANTARCTIC FISHES ADEQUATE IN THE FACE OF GLOBAL WARMING?

TUESDAY 5 JULY, 2016 10:30

• THERESA J GROVE (VALDOSTA STATE UNIVERSITY, UNITED STATES), ELIZABETH L CROCKETT (OHIO UNIVERSITY, UNITED STATES), KRISTIN M O'BRIEN (UNIVERSITY OF ALASKA, UNITED STATES), ANTHONY P FARRELL (UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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In attempting to understand the thermal tolerance of Antarctic notothenioid fishes that inhabit probably the most stenothermal environment on earth, it is important to examine physiological and biochemical effects of warming over an acute timescale (hours), as well as using temperature acclimations over longer time scales (days). One systems-level function that is thought to play a crucial role in defining the upper temperature at which vertebrates can effectively operate is the cardiovascular system. We examined the effects of a thermal ramp on heart rate ( $f_H$ ) in the red-blooded *Notothenia coriiceps* (haemoglobin and myoglobin positive) and compared this with the response of two icefish species, the white-hearted (Hb-/Mb-) *Chaenocephalus aceratus* and the red-hearted (Hb-/Mb+) *Pseudochaenichthys georgianus*. *N. coriiceps* was able to raise  $f_H$  with warming in the absence of cardiac arrhythmia to a higher temperature threshold than either of the two icefish species under investigation. We sought to identify the extent to which cardiac arrhythmia may provide a useful index of thermal tolerance (critical thermal maximum or  $CT_{MAX}$ ). Our initial findings suggest that this is the case in *C. aceratus* that attempt to minimise tachycardia by means of a high vagal tone, whereas *N. coriiceps* raises  $f_H$  in line with temperature but experiences a bradycardia just before  $CT_{MAX}$  and ventricular tachycardia just before death. These data are complemented by in situ heart preparations used to quantify maximum cardiac power output in ambient and warm-acclimated notothenioids exposed to a range of temperatures.

## A3.2 LIFE ON THE EDGE: TEMPERATURE AND Hb GENOTYPE IN-SENSITIVE O<sub>2</sub> BINDING IN ATLANTIC COD ERYTHROCYTES NEAR THEIR SOUTHERN DISTRIBUTION LIMIT

TUESDAY 5 JULY, 2016 11:10

• MICHAEL BERENBRINK (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM), SAMANTHA L. BARLOW (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM), JULIAN METCALFE (CEFAS LOWESTOFT, UNITED KINGDOM), DAVID A. RIGHTON (CEFAS LOWESTOFT, UNITED KINGDOM)

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Atlantic cod are a commercially important species believed to be threatened by warming seas near their southern, equatorward upper thermal edge of distribution. Limitations to circulatory O<sub>2</sub> transport, in particular cardiac output, and the geographic distribution of functionally different haemoglobin genotypes have separately been suggested to play a role in setting thermal tolerance in this species. The present study assessed the thermal sensitivity of O<sub>2</sub> binding in Atlantic cod red blood cells with different Hb genotypes near their upper thermal distribution limit and modelled its consequences for the arterial-venous O<sub>2</sub> saturation difference,  $S_{a-v}$ , another major determinant of circulatory O<sub>2</sub> supply rate. Results showed statistically indistinguishable red blood cell O<sub>2</sub> binding between the three HbI genotypes in wild-caught Atlantic cod from the Irish Sea (53° North). Red blood cells had an unusually low O<sub>2</sub> affinity, with reduced or even reversed thermal sensitivity between pH 7.4 and 7.9 and 5.0 and 20.0°C. This was paired with strongly pH-dependent affinity and cooperativity of red blood cell O<sub>2</sub> binding (Bohr and Root effects). Modelling of  $S_{a-v}$  at physiological pH, temperature and O<sub>2</sub> partial pressures revealed a substantial capacity for increases in  $S_{a-v}$  to meet rising tissue O<sub>2</sub> demands at 5.0 and 12.5°C, but not at 20°C. There was further no evidence for an increase of maximal  $S_{a-v}$  with temperature. It is suggested that Atlantic cod at such high temperatures may solely depend on increases in cardiac output and blood O<sub>2</sub> capacity, or thermal acclimatisation of metabolic rate, for matching circulatory O<sub>2</sub> supply to tissue demand.

### A3.3 INFLUENCE OF CORONARY BLOOD FLOW ON CARDIAC FUNCTION AND WHOLE ANIMAL THERMAL TOLERANCE IN RAINBOW TROUT

TUESDAY 5 JULY, 2016 11:25

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In approximately two thirds of all teleosts, the only route of myocardial oxygenation is via the venous oxygen supply, whereas the hearts of the remaining teleosts (e.g. salmonids) also receive oxygenated arterial blood via the coronary vasculature. Thermal tolerance is supposedly related to insufficient myocardial oxygen availability at high temperatures, but the importance of the coronary system on cardiovascular and whole animal thermal performance remains unexplored. In the current study, we investigated the influence of temperature on coronary blood flow and the effects of coronary occlusion on cardiac and whole animal thermal performance in rainbow trout, *Onchorhynchus mykiss*. Coronary occlusion in anaesthetized trout resulted in drastic changes in the electrophysiological properties of the heart including reduced R wave amplitude and an elevated ST segment in the ECG, which are both indicative of myocardial ischemia. Resting coronary blood flow was  $0.95 \pm 0.2 \text{ ml min kg}^{-1}$  at  $10^\circ\text{C}$ , but decreased to  $0.50 \pm 0.07 \text{ ml min kg}^{-1}$  following an acute thermal increase to  $18^\circ\text{C}$  (representing 7.9 and 2.7% of cardiac output, respectively). Coronary ligation in vivo resulted in earlier onset of cardiac deterioration, as indicated by reduced heart rate, during warming and a lower  $\text{CT}_{\text{max}}$  in comparison to sham treated trout ( $25.3^\circ\text{C}$  versus  $26.3^\circ\text{C}$ ). While these results indicate an important influence of the coronary blood supply on thermal tolerance and cardiovascular performance in trout, it appears that the elevated heart rate during warming may constrain coronary blood flow and hence myocardial oxygen supply when it is needed the most.

### A3.4 CAN AIR-BREATHING FISH BE ADAPTED TO HIGHER THAN PRESENT TEMPERATURES?

TUESDAY 5 JULY, 2016 11:40

MARK BAYLEY (AARHUS UNIVERSITY, DENMARK), CHRISTIAN DAMSGAARD (AARHUS UNIVERSITY, DENMARK), MIKKEL THOMSEN (AARHUS UNIVERSITY, DENMARK), MADSKUHLMANN ANDERSEN (AARHUS UNIVERSITY, DENMARK), MY LE PHUONG (AARHUS UNIVERSITY, DENMARK), DO THI THANH HUONG (CAN THO UNIVERSITY, VIETNAM), TOBIAS WANG (AARHUS UNIVERSITY, DENMARK)

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Air-breathing in fish is thought to have evolved in environments at lower than present oxygen levels and higher than present temperatures raising the question of whether extant species are adapted to recent temperature regimes, or presently living at sub-optimal temperatures. The air-breathing *Pangasionodon*

*hypophthalmus* inhabits the Mekong river system covering two climate zones during its life cycle and migrating more than 2000 km from hatching in northern Laos to its adult life in the southern delta region. It is a facultative air-breather with well-developed gills and air-breathing organ and an unusual circulatory blueprint. Here we examine the question of its optimal temperature through aspects of its cardiorespiratory physiology including temperature effects on blood oxygen binding, ventilation and blood gases, stereological measures of cardiorespiratory system, metabolic rate and growth rate. Comparing these data with present environmental temperatures throughout its distribution range, together with projected future temperatures and paleotemperatures, leads us to conclude that this species has not lost its tolerance for higher than present temperatures and that the projected temperature increases for the Mekong river *per se*, will not be detrimental to this economically important species.

### A3.5 INFLUENCE OF TEMPERATURE ON OXYGEN CONSUMPTION IN TWO KRILL SPECIES FROM THE ST. LAWRENCE ESTUARY, CANADA

TUESDAY 5 JULY, 2016 12:10

ANGÉLIQUE OLLIER (INSTITUT DES SCIENCES DE LA MER DE RIMOUSKI, CANADA), DENIS CHABOT (INSTITUT MAURICE-LAMONTAGNE, CANADA), CÉLINE AUDET (INSTITUT DES SCIENCES DE LA MER DE RIMOUSKI, CANADA), GESCHE WINKLER (INSTITUT DES SCIENCES DE LA MER DE RIMOUSKI, CANADA)

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In the context of global warming, we were interested to define the impact of temperature on physiological processes of two dominant krill species, *Meganyctiphanes norvegica* and *Thysanoessa raschii*, in the St. Lawrence Estuary (eastern Canada). These macrozooplankton are keystone species for ecosystem functioning by channeling energy from primary producers to higher trophic levels. Both species differ in their spatial and thermal habitat distribution, *M. norvegica* being a temperate species and *T. raschii* an Arctic one. We expected different optimal temperature ranges for each species and we hypothesized that metabolic and swimming rates will be directly affected by temperature conditions. The metabolic rate was measured as oxygen consumption ( $\text{MO}_2$ ). New respirometers were designed to quantify simultaneously  $\text{MO}_2$  and the swimming activity of individual krill over a period of 24h, using intermittent-flow respirometry. Significant positive regressions were obtained between  $\text{MO}_2$  and swimming speed for each species, allowing the estimation of standard and maximal metabolic rates (SMR and MMR). Swimming speed had little impact on  $\text{MO}_2$ , indicating that these continuously active species are efficient swimmers. The lowest swimming activity is observed at  $0^\circ\text{C}$ , approaching the suggested lowest critical temperature of these species. The SMR, MMR, and the aerobic scope (AS) continued to increase from  $0^\circ\text{C}$  until  $15^\circ\text{C}$ , and interestingly, there were no obvious differences between both species. Our findings will help in assessing the probable responses of both krill species to climate change.

### A3.6 DEPLETION OF OMEGA 3 IN THE FOOD SOURCE AFFECTS AEROBIC CAPACITIES OF THE GOLDEN MULLET IN A WARMING SEAWATER CONTEXT

TUESDAY 5 JULY, 2016 12:25

MARIE VAGNER (LITTORAL ENVIRONNEMENT SOCIÉTÉS - UMR 7266, FRANCE), THOMAS LACOUÉ-LABARTHE (LITTORAL ENVIRONNEMENT SOCIÉTÉS - UMR 7266, FRANCE), JOSÉ-LUIS ZAMBONINO INFANTE (IFREMER, FRANCE), DAVID MAZURAS (IFREMER, FRANCE), EMMANUEL DUBILLOT (LITTORAL ENVIRONNEMENT SOCIÉTÉS - UMR 7266, FRANCE), HERVÉ LE DELLIOU (IFREMER, FRANCE), PATRICK QUAZUGUEL (IFREMER, FRANCE), CHRISTEL LEFRANÇOIS (LITTORAL ENVIRONNEMENT SOCIÉTÉS - UMR 7266, FRANCE)

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The objective was to evaluate the combined effects of thermal acclimation and dietary n-3 highly unsaturated fatty acids (n-3 HUFA) on the aerobic capacities of the golden grey mullet *Liza aurata* in a thermal changing environment. For four months, fish were exposed to two food sources with contrasting n-3 HUFA contents (4.8% eicosapentaenoic acid EPA + docosahexaenoic acid DHA on the dry matter DM basis vs. 0.2% EPA+DHA on DM) combined with two acclimation temperatures (12°C vs. 20°C). The four experimental conditions were LH12, LH20, HH12 and HH20. Each group was then submitted to a thermal challenge consisting of successive exposures to five temperatures (9°C, 12°C, 16°C, 20°C, 24°C). At each temperature, the maximal and minimal metabolic rates, metabolic scope, and the maximum swimming speed were measured. The cost of maintenance of basal metabolic activities was particularly higher in LH groups. Moreover, LH20 exhibited a higher aerobic scope and a greater expenditure of energy to reach the same maximum swimming speed as other groups. This suggested a reduction of the amount of energy available to perform other physiological functions. This study is the first to show that the impact of lowering n-3 HUFA food content is exacerbated for fish previously acclimated to a warmer environment. It raises the question of the consequences of longer and warmer summers already recorded and still expected in temperate areas, and of the pertinence of the lowering n-3 HUFA availability in the food web expected with global change, as a factor affecting marine organisms.

### A3.7 AQUATIC DEVELOPMENT IN FISHES WITH DIFFERENT LIFE HISTORIES IN LOW OXYGEN ENVIRONMENTS

TUESDAY 5 JULY, 2016 13:40

PATRICIA A WRIGHT (UNIVERSITY OF GUELPH, CANADA)

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Metabolism in encapsulated fish embryos is dependent on diffusive rather than convective processes. The challenge of obtaining sufficient oxygen is exacerbated by an aquatic environment with low oxygen solubility and diffusivity resulting in thick boundary layers even under normoxic conditions. Hypoxic water or low flow rate further reduces oxygen availability for the embryo. Oxygen demand by the embryo increases with development exacerbating boundary layer effects. The talk will compare two fish species, the rainbow trout (*Oncorhynchus mykiss*) and zebrafish (*Danio rerio*). In the cold water, slow-developing trout, chronic hypoxia (eg. conditions that may occur in redds) during embryonic development depressed metabolism, heart rate, body movements, erythropoiesis and developmental rate. Hypoxia-reared larvae also had three- to six-fold higher mRNA expression of the embryonic Hb  $\alpha$ -1,  $\beta$ -1 and  $\beta$ -2 subunits relative to stage-matched normoxia-reared larvae. Hypoxia also altered the regulatory control of heart rate in trout in a stage-dependent manner. In warm water, fast-developing zebrafish, acute hypoxia (4h, typical of tropical ponds) during embryogenesis induced the hypoxia inducible factor (HIF)-1 cellular response resulting in larvae with enhanced hypoxia tolerance and adults with altered sex ratios. The critical window for hypoxia sensitivity and HIF-1 signalling in zebrafish was 24 hours post fertilization. Comparisons between physiological responses to early hypoxia and life history strategies will be discussed.

### A3.8 ARE THERE LONG TERM EFFECTS OF DEVELOPMENTAL HYPOXIA ON THE METABOLIC PHYSIOLOGY OF ATLANTIC SALMON?

TUESDAY 5 JULY, 2016 14:20

ANDREW T WOOD (UNIVERSITY OF TASMANIA, AUSTRALIA), TIMOTHY D CLARK (UNIVERSITY OF TASMANIA, AUSTRALIA), SARAH J ANDREWARTHA (CSIRO, AUSTRALIA), NICHOLAS G ELLIOTT (CSIRO, AUSTRALIA), PETER B FRAPPELL (UNIVERSITY OF TASMANIA, AUSTRALIA)

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Developmental hypoxia (oxygen deficiency) can potentially impact long-term physiological performance of fish due to irreversible plasticity. Atlantic salmon (*Salmo salar*) can experience hypoxia during development in natural salmon redds or when raised in a hatchery environment. Physiological plasticity may influence performance through its effect on metabolic rates and subsequently swimming performance, growth or survival. We investigated the long term impacts of developmental hypoxia by incubating salmon embryos and alevins in 50% dissolved oxygen (% of air saturation; PO<sub>2</sub> ~10kpa) for three months and then raising them in normoxic (100% dissolved oxygen; PO<sub>2</sub> ~21kpa) conditions for a further 15 months. Aerobic scope was calculated as the difference between

minimum and maximum oxygen uptake rates ( $MO_{2min}$  and  $MO_{2max}$ , respectively) in hypoxia and normoxia. Hypoxia tolerance was determined by measuring the dissolved oxygen level at loss of equilibrium in a constantly declining oxygen environment. We found no long-term effect of developmental hypoxia on  $MO_{2min}$ ,  $MO_{2max}$  or aerobic scope in hypoxia or normoxia following 15 months of rearing in normoxia. However, there was some evidence that tolerance to hypoxia was lower in salmon exposed to developmental hypoxia, although the effect size was small. Few studies have investigated such long-term impacts of developmental hypoxia in fish. Future research aims to investigate the immediate and short term impacts of developmental hypoxia on salmon physiology.

### A3.9 EFFECTS OF INCREASED TEMPERATURE DURING CRITICAL WINDOWS OF DEVELOPMENT ON EMBRYONIC AND HATCHING LAKE WHITEFISH PHENOTYPES

TUESDAY 5 JULY, 2016 14:35

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An animal's developmental trajectory is a result of interactions between genome and the environment. The ability to modify phenotype via plasticity allows embryonic animals to cope with challenges during development, including altered environmental conditions. Critical windows are periods during embryonic development and/or early life when phenotypes are particularly plastic and responsive to intrinsic or extrinsic (environmental) factors. Using embryonic lake whitefish (*Coregonus clupeaformis*), we explored the effects of increased and variable incubation temperature on embryonic and hatching phenotypes. Lake whitefish are cold-water developers (<10°C) that may be subjected to increased incubation temperatures from climate change and anthropogenic sources. We examined plasticity in the survival, development rate, growth, heart rate, and energy use of embryos following incubation in warm temperatures during distinct periods of embryonic development. Organogenesis is a sensitive period for embryonic oxygen consumption rate and heart rate, and the energetic cost of development (yolk uptake and oxygen consumption) is altered by temperature during the late growth period of development when energy demands are highest. We also examined hatching survival, size and oxygen consumption rate to assess if hatching phenotypes are influenced by embryonic conditions. Increased temperature during the last 30% of development triggered early hatching when body mass is lower, and this may impact post-hatching survival, growth and performance.

### A3.10 PROVISION OF OXYGEN TO DEVELOPING EMBRYOS OF THE SNAPPING TURTLE *CHELYDRA SERPENTINA*

TUESDAY 5 JULY, 2016 14:50

MARINA R. SARTORI (UNIVERSITY OF SAO PAULO STATE, BRAZIL), ZACHARY F. KOHL (UNIVERSITY OF NORTH TEXAS, UNITED STATES), AUGUSTO S. ABE (UNIVERSITY OF SAO PAULO STATE, BRAZIL), DANE A. CROSSLEY II (UNIVERSITY OF NORTH TEXAS, UNITED STATES), EDWIN W. TAYLOR (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM)

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We have measured the variables determining the provision of oxygen to tissues of developing embryos of the snapping turtle, *Chelydra serpentina*. Measured variables included: heart rate ( $f_H$ ); oxygen consumption ( $VO_2$ ) and arterio-venous oxygen difference (A-V diff). Using the Fick equation we calculated cardiac output (Q). Relative blood flow (%Q) to the embryo and to the chorioallantoic membrane (CAM) was measured using microspheres. In vitro techniques on blood samples provided  $O_2$  carrying capacity ( $Ca_{Tot} O_2$ );  $O_2$  affinity curves to yield  $P_{50}$ ; and hemoglobin content [Hb]. We measured these variables in embryos at 50, 70, and 90% of the incubation period. Increase in body mass is paralleled by an increase in the mass of the heart and increased metabolic rate, measured as oxygen consumption.  $f_H$  did not change from 50% to 70% of incubation but was significantly reduced at 90%. [Hb] did not change but A-V diff doubled from 50 to 90% of incubation.  $P_{50}$  values revealed an increased affinity under 2%  $CO_2$  and a decreased affinity under 6%  $CO_2$  at 70 and 90% incubation. %Q to the embryo decreased from 50 to 70% incubation whereas it increased to the CAM that provides the surface for respiratory gas exchange across the eggshell. We conclude that embryos rely on an optimized binding of oxygen to Hb across the CAM and release of  $O_2$  to the embryonic tissues during the latter stages of incubation, possibly ensured by different isoforms or allosteric effects on the hemoglobin.

### A3.11 QUANTITATIVE TRANSCRIPTOMICS REVEALS THE IMPORTANCE OF COLD ACCLIMATION FOR PROLONGED ANOXIA SURVIVAL IN *TRACHEMYS SCRIPTA*

TUESDAY 5 JULY, 2016 15:05

JONATHAN A.W. STECYK (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES), THIRU RAMARA (NATIONAL CENTER FOR GENOME RESOURCES, UNITED STATES), JOHNNY SENA (NATIONAL CENTER FOR GENOME RESOURCES, UNITED STATES), FAYE SCHILKEY (NATIONAL CENTER FOR GENOME RESOURCES, UNITED STATES)

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The heart of the red-eared slider turtle (*Trachemys scripta*) can continue to beat rhythmically during anoxia, albeit more slowly. A dramatic and rapid resetting of the intrinsic pacemaker contributes to the bradycardia, but the underlying mechanisms remain unknown. We employed next-generation sequencing and

quantitative transcriptomics to discover how the *T. scripta* sinoatrial node is remodelled with acclimation to low temperature and anoxia exposure. Total RNA was extracted from the sinus venosus of turtles exposed to normoxia at 21°C, 24 h of anoxia at 21°C, normoxia at 5°C or 14 d of anoxia at 5°C ( $N=3$ ) and utilized for Illumina RNA library preparation, Illumina sequencing, *de novo* Illumina transcript assembly and differential transcript expression analysis. Differentially expressed (DE) transcripts were selected as those with a posterior probability differential expression (PPDE) greater or equal to 0.95. In stark contrast to anoxia exposure at 21°C, which was associated with the DE of 4921 transcripts, anoxia exposure at 5°C was only associated with the DE of 860 transcripts. Moreover, only 62 transcripts were similarly affected by anoxia at 21°C and 5°C. However, acclimation to 5°C in normoxia induced the DE of 23567 transcripts. These transcripts included approximately 50% of those DE by anoxia at 21°C. Combined, the findings suggest that altered transcript expression with cold acclimation primes the turtle cardiac muscle for the approaching anoxic winter, whereas cardiac anoxia survival at 21°C is aided by the circumvention of this priming and through the induction of similar changes in transcript expression.

### A3.24 TEMPERATURE DEPENDENT EFFECTOR BINDING: THE DEFINITIVE MOLECULAR MECHANISM UNDERLYING HEMOGLOBIN THERMAL SENSITIVITY

TUESDAY 5 JULY, 2016 16:00

KEVIN L CAMPBELL (UNIVERSITY OF MANITOBA, CANADA), ANTHONY V SIGNORE (UNIVERSITY OF MANITOBA, CANADA), PHILLIP R MORRISON (UNIVERSITY OF BRITISH COLUMBIA, CANADA), COLIN J BRAUNER (UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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As hemoglobin's affinity for  $O_2$  is inversely related to temperature, Arctic species may experience reduced  $O_2$  offloading to poorly insulated appendages. However, select species have evolved hemoglobin proteins with reduced thermal sensitivity that can maintain  $O_2$  delivery at low temperatures. This phenotype has historically been attributed to the binding of additional allosteric effectors to the hemoglobin moiety relative to that of non-cold adapted species. Conversely, recent evidence indicates that hemoglobin from the extinct Steller's sea cow binds fewer allosteric effectors than those of its tropical relatives (dugongs and manatees), yet surprisingly, the presence of these ligands reduces its thermal sensitivity to a greater extent. To elucidate the mechanisms underlying this phenomenon, we measured the  $O_2$  affinity of woolly mammoth, elephant, and sirenian hemoglobins in the presence of serially increasing allosteric effector concentrations at both 25 and 37°C. Quantitation of effector binding revealed that total effector binding (i.e., number of effector molecules bound) is not directly linked to the thermal sensitivity of hemoglobin. Rather, effector binding is revealed to be temperature dependent (i.e., it increases as temperature decreases), with cold adapted hemoglobins exhibiting greater increases in relative effector binding - and hence, a lower thermal sensitivity - than those of non-cold adapted species.

### A3.25 FUNCTIONAL DIVERSIFICATION OF RETINAL OXYGEN SUPPLY IN BONY FISHES

TUESDAY 5 JULY, 2016 16:30

CHRISTIAN DAMSGAARD (AARHUS UNIVERSITY, DENMARK), HENRIK LAURIDSEN (AARHUS UNIVERSITY, DENMARK), ANETTE M.D. FUNDER (AARHUS UNIVERSITY, DENMARK), JESPER S. THOMSEN (AARHUS UNIVERSITY, DENMARK), ANNEMARIE BRÜEL (AARHUS UNIVERSITY, DENMARK), DO T.T. HUONG (CAN THO UNIVERSITY, VIETNAM), NGUYEN T. PHUONG (CAN THO UNIVERSITY, VIETNAM), 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 fish retina is avascular with a high metabolic demand requiring a well-developed oxygen supply. In basal ray-finned fishes, the retina receives oxygen through pre-retinal capillaries on the inner side of the retina, while higher teleosts secrete oxygen from a *choroid rete* via Root effect hemoglobins. To investigate the hypothesis that the evolutionary emergence of oxygen secretion allowed for a thicker retina and hence higher visual acuity, we quantified the anatomy of the retina, *choroid rete* and pre-retinal capillaries using stereological principles with histological sections, ultrasound and computed tomography from eyes of 35 species of bony fishes. These morphological characters were combined with measures of hemoglobin Root effect to reconstruct the evolutionary interplay between retinal oxygen supply and retinal anatomy. We show that retina thickness doubled after the origin of oxygen secretion, demonstrating the efficiency of oxygen secretion in retinal oxygen delivery. The *choroid rete* was secondarily lost 9 independent times. Here, retinal thickness was halved and pre-retinal capillaries took over retinal oxygen supply. Also, these capillaries were well developed in basal teleosts even after the origin of oxygen secretion, and were lost before the split of *Onchorhynchus* and *Percomorpha*, which was linked to an increase in *choroid rete* surface area. Lastly, we demonstrate that *choroid rete* surface area was secondarily reduced in the ancestor of *Symbranchiformes*, where pre-retinal capillaries re-evolved. This study shows how the functional elements underlying retinal oxygen supply interplay, and how the diversity of oxygen delivery solutions shaped retinal anatomy on an evolutionary time scale.

### A3.26 NO VALVES REQUIRED: TUBULAR HEART PUMPING MECHANISMS IN TUNICATES

TUESDAY 5 JULY, 2016 16:45

NICHOLAS A BATTISTA (UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL, UNITED STATES)

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In vertebrate embryogenesis, the first organ to form is the heart, beginning as a primitive heart tube. However, many invertebrates have tubular hearts from infancy through their adulthood. Heart

tubes have been described as peristaltic and impedance pumps. Impedance pumping assumes a single actuation point of contraction, while traditional peristalsis assumes an active traveling wave of actuation. In addition to differences in flow, this inherently implies differences in the conduction system. It is possible to transition from pumping mechanism to the other with a change in the diffusivity of the action potential. In this work we consider the coupling between the fluid dynamics and electrophysiology of both mechanisms, within a basal chordate, the tunicate. Using CFD with an electromechanical model of tubular pumping, we discuss implications of the both mechanisms. Furthermore, we discuss the implications of the pumping mechanism on evolution and development.

### A3.27 HAEMOGLOBIN ADAPTATIONS TO SUBTERRANEAN BURROWS: LESSONS FROM AFRICAN MOLE RATS

TUESDAY 5 JULY, 2016 17:00

ROY E. WEBER (AARHUS UNIVERSITY, DENMARK), JENNIFER U.M. JARVIS (UNIVERSITY OF CAPE TOWN, SOUTH AFRICA), ANGELA FAGO (AARHUS UNIVERSITY, DENMARK), NIGEL C. BENNETT (UNIVERSITY OF PRETORIA, SOUTH AFRICA)

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Mole rats are strictly subterranean rodents that exhibit a range of striking anatomical, behavioural and physiological specializations, including the capacity to live and carry out intense burrowing activity under extremely hypoxic and hypercapnic conditions ( $O_2$  tensions lower than at the summit of Mount Everest and burrow-air  $CO_2$  concentrations above 6%). Species of African (family Bathyergidae) mole rats, which may be solitary or eusocial (living in large families with a single breeding pair) and show extreme longevity, inhabit intricate, deep and sealed subterranean burrows under a wide variety of conditions as regards soil types, relative humidity and vegetation diversity. Compared to the high altitude mammals, little is known about the adaptations in haemoglobin (Hb) function that secure the exchange and internal transport of respiratory gases. With the view of identifying the implicated cellular and molecular mechanisms we report haematological parameters, as well as Hb- $O_2$  binding characteristics, viz., the intrinsic Hb- $O_2$  affinities and their sensitivities to pH and  $CO_2$  (the 'fixed acid' and ' $CO_2$ ' Bohr effects), 2,3 diphosphoglycerate (DPG, the main organic modulator of  $O_2$  affinity in mammalian red blood cells), and temperature, in six species of African mole rats that differ in sociality and biome and soil types. Our study reveals slight differences in haematological characters (haematocrit and red cell Hb and DPG concentrations) and intrinsic  $O_2$  affinities that were not clearly correlated with sociality or soil type, but marked reductions in specific (pH-independent)  $CO_2$  sensitivity that may contribute to safeguarding pulmonary  $O_2$  loading in hypoxic-hypercapnic burrows.

### A3.12 ELECTRON TOMOGRAPHY OF AVIAN CARDIAC CALCIUM RELEASE UNITS

TUESDAY 5 JULY, 2016 POSTER SESSION

THOMAS SHEARD (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

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Bird ventricular myocytes are long, thin and lack T-tubules, which is in line with myocytes from fish, amphibians and reptiles. However, bird hearts achieve higher contractile rates and are capable of stronger pressure development than many mammals and all ectothermic species. How do they achieve this at a cellular level? Excitation-contraction coupling in vertebrate hearts is underpinned by calcium diffusion between the calcium release units (CRUs), formed by clusters of ryanodine receptors present on the surface of the sarcoplasmic reticulum (SR). In birds, calcium released at the sarcolemma at peripheral couplings must diffuse to internal corbular SR structures for activation propagation, with no T-tubular network of dyads to facilitate spread of calcium release. E-C coupling may be limited when distances between these structures are greater than the limitations for calcium diffusion allow. Distances between CRUs are therefore crucial in determining whether further calcium-induced calcium release (CICR) occurs.

This project uses electron tomography to study chicken cardiac muscle and characterise the sarcoplasmic reticulum network in 3-D. Previous data for distances between CRUs in birds uses transmission electron microscopy (TEM) in the left ventricle. TEM is not optimal for accurate analysis of 3D structural interplay. Tomography is able to provide information on the distances between structures, as well as volumes. Thus far, reconstruction of cardiac myocytes with tomography demonstrates the pathways for calcium diffusion throughout the cell. We report strings of corbular SR CRUs found at the Z-lines which link to regions of extracellular Ca influx at peripheral couplings.

### A3.13 CHARACTERIZATION OF THE GENETIC MECHANISMS RESPONSIBLE FOR ADAPTATION TO HYPOXIC STRESS DURING BROILER EMBRYOGENESIS

TUESDAY 5 JULY, 2016 POSTER SESSION

AMIT HARON (ARO - HEBREW UNIVERSITY, ISRAEL), ZVI PELEG (HEBREW UNIVERSITY, ISRAEL), DRUYAN SHELLY (ARO, ISRAEL)

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Environmental alteration during development of an organism may alter development of some physiological regulatory systems and induce permanent phenotypic changes in the chick embryo.

The purpose of this study was to investigate the genetic adaptive responses of the chick heart to hypoxic challenges (17%  $O_2$ ) during the plateau phase.

RNA was extracted from hearts of commercial broiler embryos that were subjected to 17% hypoxia on embryonic day 16 (E16). The transcriptome of the hearts was formed after RNA-seq and expression of the genes was compared to a control group in order to

find pathways that contribute to the embryos' adaptive response to hypoxia.

In 530 genes, differential expression patterns were identified between hypoxic and control hearts. Those genes were associated with many different pathways including response to stress, regulation of growth, and proteolysis. In two different timepoints, processes occurring in the mitochondria, such as respiratory chain, were found to differ between the hypoxic and control groups.

Our findings suggest that the cellular metabolic and respiratory activity in the embryos decrease after 2 hours of hypoxia, and later on (after 8 hours) there was an adaptation, indicated by elevated activity, allowing supply of the energy that was lost due to the first response.

Better understanding of the embryos' response to hypoxia, will allow us to determine the exact incubation conditions for optimal programming of the regulatory systems of broiler embryos in order to improve their post hatch performance under sub-optimal conditions.

### A3.14 VENTILATORY AND CARDIOVASCULAR REGULATION IN THE AIR-BREATHING FISH *PANGASIANODON HYPOPHTHALMUS*

TUESDAY 5 JULY, 2016 POSTER SESSION

MIKKEL THOMSEN (ZOOLOGY DEPARTMENT OF BIOSCIENCE AARHUS UNIVERSITY, DENMARK), TOBIAS WANG (ZOOLOGY DEPARTMENT OF BIOSCIENCE AARHUS UNIVERSITY, DENMARK), MARK BAYLEY (ZOOLOGY DEPARTMENT OF BIOSCIENCE AARHUS UNIVERSITY, DENMARK)

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The air-breathing fish *Pangasianodon hypophthalmus* is abundant in the Mekong river system where it is also intensively cultured. In contrast to most other air-breathing fishes it has well developed gills as well as a highly trabeculated swim bladder with a large surface area used for air-breathing. Its native waters have been shown to be periodically strongly hypoxic and hypercarbic, forcing *P. hypophthalmus* to switch from exclusively branchial ventilation to air-breathing to maintain its aerobic metabolism. This ability to switch respiratory media demands that the oxygen- and CO<sub>2</sub> sensor systems provide information on when gill ventilation is insufficient for oxygen uptake and hence initiate air-breathing. Here we investigate the ventilatory and cardiovascular responses to changes in either in the external media or internally in the blood in resting fish. We found ventilation in *P. hypophthalmus* to be unaffected by aquatic CO<sub>2</sub> from 0 to 5%, whereas moderate hypoxia stimulated branchial ventilation while initiating air-breathing at low frequencies. *P. hypophthalmus* switched to almost exclusive air-breathing with minimal branchial ventilation in severe hypoxia. We observed a hypoxic bradycardia and a post air-breathing tachycardia as well as a hypercarbic bradycardia. Furthermore, we document that injections of lactate ions in the blood at physiological concentrations induce a strong hypoxic ventilatory response in *P. hypophthalmus* independent of arterial pH. This mechanism was recently suggested to form part of the oxygen-sensing stimulus in mammals (Chang et al. 2015) and we present the first evidence of this mechanism in fish.

### A3.15 ACTION POTENTIAL FREQUENCY OF PACEMAKER CELLS DOES NOT LIMIT HEART RATE IN BROWN TROUT AT HIGH TEMPERATURES

TUESDAY 5 JULY, 2016 POSTER SESSION

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Temperature-induced increase in cardiac output (CO) in fish is almost totally dependent on increases in heart rate (HR) and at high temperatures CO collapses due to the deterioration of HR. This study tests the hypothesis that firing rate of cardiac pacemaker cells (pacemaker rate, PR) limits HR in fish at high temperatures. To this end temperature-dependence of action potential (AP) frequency of enzymatically isolated pacemaker cells and spontaneous beating rate of isolated sinoatrial preparations of the cold-acclimated (+4°C) brown trout (*Salmo trutta fario*) were compared under acute heat ramps. Rising temperature increased PR due to the acceleration of diastolic depolarization and shortening of AP duration. The peak PR was much higher (158 ± 21 beats per minute (bpm) at 26.6°C) than the peak HR of sinoatrial preparations (75.3 ± 4.7 bpm at 23.7°C). In the presence of 5 nM isoprenaline the maximum PR and HR were 158 ± 24 (at 26.5°C) and 94.7 ± 8.5 bpm (24.6°C), respectively. These findings strongly suggest that in brown trout the maximum HR is not limited by the frequency of pacemaker APs, but by the inability of the atrial tissue to follow the rate of the pacemaker. The failure is not in the impulse generation, but in the impulse transmission from the pacemaker to the atrium.

### A3.16 HEMOGLOBIN GENE SYSTEM IN EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*): GENOMIC ORGANIZATION AND GENE EXPRESSION PATTERN IN DIFFERENT ENVIRONMENTAL CONDITIONS

TUESDAY 5 JULY, 2016 POSTER SESSION

LAURA CADIZ (IFREMER, FRANCE), JOSÉ ZAMBONINO-INFANTE (IFREMER, FRANCE), ARIANNA SERVILI (IFREMER, FRANCE), PATRICK QUAZUGUEL (IFREMER, FRANCE), LAURIANE MADEC (IFREMER, FRANCE), ØIVIND ANDERSEN (NOFIMA, NORWAY), ERICK DESMARAIS (INSTITUT DES SCIENCES DE L'ÉVOLUTION, FRANCE), DAVID MAZURAI (IFREMER, FRANCE)

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Climate projections expect the increase in global average temperature and in the frequency and intensity of hypoxia events. These environmental constraints could induce acclimation in fish including the modulation of hemoglobin system involved in oxygen transport. Characterization of hemoglobin gene system including ontogeny and tissue expression pattern in different teleost species would allow better understanding and predicting their capacity to cope to environmental variations in the context of global change. In

the present study is reported for the first time the characterization of the European sea bass (*Dicentrarchus labrax*) hemoglobin system, including genomic organization and analysis of gene sequences. Genomic analysis showed that hemoglobin genes were separated into two unlinked clusters, the 'MN' cluster containing eleven genes (5  $\alpha$ -hemoglobin and 6  $\beta$ -hemoglobin) and the LA cluster consisting of three genes (2  $\alpha$ -hemoglobin and 1  $\beta$ -hemoglobin). Moreover, the impact of a moderated hypoxia episode (40% of air saturation) on the expression patterns of European sea bass hemoglobin genes were investigated during larval development (between 28 and 50 days post-hatching) at two thermal conditions (15 and 20°C). Our results show that some, but not all, hemoglobin genes were drastically up regulated under reduced dissolved oxygen levels and increased temperature, suggesting that hemoglobin system is involved in fish response to these environmental constraints. Ongoing analyses seek to determine whether the regulations of hemoglobin gene expression pattern revealed in larvae persist into later life stages and impact juvenile performance in constraining environments.

### A3.17 THE SECRET BEHIND AN INCREASED STROKE VOLUME IN SEAWATER-ACCLIMATED RAINBOW TROUT

TUESDAY 5 JULY, 2016 POSTER SESSION

ESMÉE DEKENS (HU UNIVERSITY OF APPLIED SCIENCES UTRECHT, NETHERLANDS), JEROEN BRIJS (UNIVERSITY OF GOTHENBURG, SWEDEN), MICHAEL AXELSSON (UNIVERSITY OF GOTHENBURG, SWEDEN), ERIK SANDBLOM (UNIVERSITY OF GOTHENBURG, SWEDEN)

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The rainbow trout (*Oncorhynchus mykiss*) is a euryhaline teleost that can acclimate easily to a wider range of salinities through various morphological, physiological and behavioural modifications. A recent study showed that rainbow trout increase gut blood flow two-fold during seawater acclimation. The increase in gut blood flow was associated with an elevated cardiac output, primarily due to an increased stroke volume while heart rate remained unchanged. However, the mechanisms behind the increase in stroke volume during seawater acclimation remain unknown. Stroke volume can either increase due to increased ventricular size, improved cardiac contractility, increased venous filling pressure or a combination of these factors. To investigate the importance of each of these factors we measured ventricular mass, ratio of compact and spongy myocardium and venous filling pressures of freshwater- and seawater-acclimated trout. Preliminary results indicate that although relative and absolute ventricular masses remain unchanged, cardiac remodelling occurs in seawater-acclimated trout with significant increases in the proportion of compact myocardium suggesting that ventricular contractility may be improved. Recordings of venous filling pressures at different salinities are currently ongoing and the results will be presented and further discussed.

### A3.18 CHILLED OUT VASOACTIVITY IN ANTARCTIC ICEFISH

TUESDAY 5 JULY, 2016 POSTER SESSION

THERESA J GROVE (VALDOSTA STATE UNIVERSITY, UNITED STATES), ELIZABETH L CROCKETT (OHIO UNIVERSITY, UNITED STATES), KRISTIN M O'BRIEN (UNIVERSITY OF ALASKA, UNITED STATES), STUART EGGINTON (UNIVERSITY OF LEEDS, UNITED KINGDOM)

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Life in the cold poses a number of challenges for vertebrates with a cardiovascular system normally associated with maximising delivery of oxygen. Constraints of heart muscle and vascular smooth muscle function dictates a likely compromise between cardiac output, tissue perfusion, and blood pressure regulation. The lack of facilitated oxygen transport in haemoglobinless icefish (Channichthyidae) suggests that the system is likely a perfusion rather than diffusion limited system in terms of oxygen transport to tissue. Given the unusually large hearts that deliver an impressive blood flow, this implies a low vascular resistance. Estimates of vessel dimensions support this contention but until now no estimates of vascular function from these animals has been possible. Using wire myography to determine the reactivity of branchial efferent vessels, which regulate systemic flow of oxygenated blood from gills, we examined the origin of low vascular resistance in these animals. Icefish showed a similar sensitivity to electrogenic (KCl) constriction relative to red-blooded species. While an attenuated vasoconstrictor profile was to be expected (NA<sub>2</sub>, AngII, 5-HT, ET-1), the lack of enhanced vasodilator capacity (ACh, isoprenaline, and various NO mimetics) suggests that icefish operate with little control over vascular resistance, and hence cardiac afterload, suggesting limited capacity to adjust to additional environmental challenges.

### A3.19 THERMAL REMODELING OF CELLULAR ENERGETICS IN THE ECTOTHERM HEART

TUESDAY 5 JULY, 2016 POSTER SESSION

JOHN C MARRIN (THE UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

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Seasonal temperature change can cause a remodelling of multiple aspects of the ectothermic heart. The heart normally functions by producing energy in the form of ATP via fatty acid oxidation. However, increasing cardiac demand or inducing low oxygen conditions can result in an increased reliance on glycolytic pathways. In order to assess the remodeling of cellular energetics, rainbow trout (*Oncorhynchus mykiss*) and cold dormant freshwater turtles (*Trachemys scripta scripta*) at temperatures to simulate seasonal temperature change and used histological techniques to assess tissue content of lipid and glycogen. The results for the cold acclimated *O. mykiss* showed an increase in lipid content and decrease in glycogen. The opposite was true for warm acclimated *O. mykiss*. Conversely, for cold acclimated *T. scripta* there was an observable increase in glycogen content and a decrease in lipid content. These findings suggest that cold-induced hypertrophy in rainbow trout is facilitated by increased fatty acid oxidation;

A warm induced conversion to glycolysis highlights the stress of warm temperatures on the fish heart. The metabolic shift from fatty acid oxidation to glycolysis evident in *T. scripta* is consistent with the anoxic conditions these animals experience during winter and suggest that cold temperature acts to prime the heart for winter hibernation.

### A3.20 MITOCHONDRIAL FUNCTION MAY CONTRIBUTE TO THERMAL TOLERANCE OF RED- AND WHITE-BLOODED NOTOTHENIROID FISHES

■ TUESDAY 5 JULY, 2016 POSTER SESSION

● KRISTIN M O'BRIEN (UNIVERSITY OF ALASKA FAIRBANKS, UNITED STATES)

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Red-blooded Antarctic notothenioids have higher critical thermal maxima (CT<sub>Max</sub>) and maintain cardiac function at higher temperatures than hemoglobinless Channichthyid icefishes. To determine if mitochondrial function contributes to thermal tolerance, state 2, 3, 4 and uncoupled (ETS) respiration rates, the activity of cytochrome oxidase (CCO) and the respiratory control ratio (RCR) were measured at 2°C and 10°C in mitochondria isolated from heart ventricles of the red-blooded *Notothenia coriiceps* and icefishes *Chaenocephalus aceratus* and *Pseudochannichthys georgianus* held at ambient temperature and exposed to their CT<sub>Max</sub>. Mitochondrial proteins were also identified and quantified with mass spectrometry. State 3 and ETS rates were higher in species exposed to their CT<sub>Max</sub> compared to animals held at ambient temperature, but states 2 and 4 (leak) were unaffected. The activity of CCO and the ratio of CCO:state 3 respiration rate were significantly higher in *N. coriiceps* compared to icefishes, suggesting *N. coriiceps* may have a greater potential to increase flux through the respiratory chain when needed, and enhance cardiac function at elevated temperature. Differences in the mitochondrial proteome and potential impacts on mitochondrial function will also be discussed.

### A3.21 ANTARCTIC NOTOTHENIROID FISHES, *CHAENOCEPHALUS ACERATUS* AND *NOTOTHENIA CORIICEPS*, VARY IN METABOLITE PROFILES WHEN EXPOSED TO ACUTE THERMAL STRESS

■ TUESDAY 5 JULY, 2016 POSTER SESSION

● ELIZABETH R EVANS (OHIO UNIVERSITY, UNITED STATES), KRISTIN M O'BRIEN (UNIVERSITY OF ALASKA FAIRBANKS, UNITED STATES), ELIZABETH L CROCKETT (OHIO UNIVERSITY, UNITED STATES)

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Members of the haemoglobin-less family of notothenioid fishes, Channichthyidae, are largely endemic to the region of the Western Antarctic Peninsula, which is experiencing rapid climatic warming. Previous studies have shown that channichthyids (known as 'icefishes') have reduced thermal tolerance compared to red-blooded notothenioids. To better understand what limits

thermal tolerance, we quantified levels of glucose in blood plasma, and brain contents of glycogen, lactate, and  $\beta$ -hydroxybutyrate in white-blooded *Chaenocephalus aceratus* and red-blooded *Notothenia coriiceps* at both ambient and critical thermal maximum (CT<sub>max</sub>) temperatures. Plasma glucose is unchanged with exposure to elevated temperatures in *C. aceratus*, but increases by 4-fold in *N. coriiceps*. Brain glycogen is nearly 15x higher in *C. aceratus* than *N. coriiceps* at ambient temperature, while at CT<sub>max</sub>, glycogen is reduced by 50% in *N. coriiceps* and completely exhausted in *C. aceratus*. At ambient temperatures, lactate is 50% higher in *N. coriiceps* than *C. aceratus*, and at CT<sub>max</sub>, lactate in the brain accumulates 3-fold in *C. aceratus* and increases by a factor of 4 in *N. coriiceps*. No significant difference in  $\beta$ -hydroxybutyrate is found in either species when exposed to acute thermal stress. Our results indicate that at elevated temperatures, the icefish *C. aceratus* may have a more limited capacity to mobilize carbohydrate from hepatic sources to support brain metabolism than red-blooded *N. coriiceps*. Supported by NSF ANT 1341602.

### A3.22 GENE EXPRESSION OF CA<sup>2+</sup>, NA<sup>+</sup> AND K<sup>+</sup> CHANNELS AND PUMPS IN THE ALASKA BLACKFISH (*DALLIA PECTORALIS*) VENTRICLE AT HIGH AND LOW ACCLIMATION TEMPERATURE

■ TUESDAY 5 JULY, 2016 POSTER SESSION

● CHRISTINE S. COUTURIER (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES), ANGELA VOGT (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES), JONATHAN A.W. STECYK (UNIVERSITY OF ALASKA ANCHORAGE, UNITED STATES)

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The Alaska blackfish is the only air-breathing fish to inhabit Arctic regions. The fish presumably utilizes air-breathing in summer to support reproduction and migration to waters that are dense in vegetation, have little mixing and are hypoxic. However, in the winter, the Alaska blackfish is forcibly submerged in hypoxic water beneath the ice, which precludes air-breathing. Nevertheless, it remains active. We know from previous studies that with cold-acclimation, Alaska blackfish heart rate and ventricular Na<sup>+</sup>-K<sup>+</sup>-ATPase activity are reduced and action potential duration prolonged (Q<sub>10</sub> values of ~2 to 4). Concurrently, peak I<sub>Ca</sub> density is markedly down-regulated (Q<sub>10</sub> of 8). In order to gain insight into the regulation of action potential generation and excitation-contraction coupling at the molecular level, Alaska blackfish were acclimated to 15°C or 5°C and gene expression of Ca<sup>2+</sup>, Na<sup>+</sup> and K<sup>+</sup> channels and pumps quantified by real-time RT-PCR. Total RNA did not differ between acclimation temperature (P = 0.1157), and of the 15 transcripts measured, 3 exhibited a significantly (P < 0.05) increased expression at 5°C. Gene expression of the G protein-activated inward rectifier K<sup>+</sup> channel (KCNJ3), IKr producing rapid voltage gated (delayed rectifier; KCNH2) K<sup>+</sup> channel and solute carrier family 8, member 1 (SLC8A1; Na<sup>+</sup>/Ca<sup>2+</sup> exchanger) was increased by 1.8- to 3.2-fold. Future electrophysiological studies will investigate if the changes in gene expression correlate with functional changes in ion channel activity and conductance.

### A3.23 THE EFFECT OF ACUTE TEMPERATURE CHANGE ON THE CARDIAC PERFORMANCE OF ACTIVE PELAGIC PREDATORS

TUESDAY 5 JULY, 2016 POSTER SESSION

KARLINA OZOLINA (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), THOMAS SHEARD (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), DIEGO BERNAL (UNIVERSITY OF MASSACHUSETTS, UNITED STATES), CHUGEY SEPULVEDA (PFLEGER INSTITUTE OF ENVIRONMENTAL RESEARCH, UNITED STATES), PETER G BUSHNELL (INDIANA UNIVERSITY SOUTH BEND, UNITED STATES), HOLLY A SHIELS (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

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Fish that are exposed to acute temperature changes in their environment must possess a cardiovascular system that is able to deliver oxygen at rates that equal oxygen demands at all levels of activity (from resting to foraging, to high speed prey pursuit). Previous studies on tuna have shown temperature specific physiological adaptations (e.g. cold induced bradycardia), however comparative studies on other pelagic fishes are lacking. The aim of this study was to investigate what happens to the cardiac contractility of swordfish, bigeye thresher sharks, and blue sharks during acute temperature change. We designed an in vitro cooling and warming protocol to simulate the temperature the heart would be exposed to during a deep feeding dive and resurface. Force frequency relationship of myocardial preparations was measured at 8°C and 20°C (deep sea and surface water temperatures, respectively) in the presence of low (5nM) and high (1µM) adrenaline. Our data show a typical increase in force of contraction with decrease in temperature, however the severity of this relationship is species specific. Ultimately, understanding how physiological adaptations may limit the distribution of these large, elusive, highly active predators that roam the open ocean is of utmost importance for ecosystem based fisheries management.

# A4 THE ROLE OF INDIVIDUAL VARIATION IN THE BEHAVIOUR OF ANIMAL GROUPS

ORGANISED BY: DR SHAUN KILLEN (UNIVERSITY OF GLASGOW, UNITED KINGDOM) & DR STEFANO MARRAS (IAMC-CNR, ITALY)

## A4.1 FROM INDIVIDUALS TO GROUPS: HOW BEHAVIOUR AND PHYSIOLOGY SHAPE COLLECTIVE BEHAVIOUR

📅 MONDAY 4 JULY, 2016 ⌚ 11:00

👤 ASHLEY WARD (UNIVERSITY OF SYDNEY, AUSTRALIA)

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Collective behaviour is most clearly expressed when many animals act coherently and synchronously, apparently conforming to the behaviour of their near neighbours and behaving globally as if of one mind. Set against this apparent uniformity of behaviour in a social context, the paradigm of personality, or consistent individual differences among animals, is well established in the field of animal behaviour. Similarly, we know that physiology, a major driver of animal behaviour, differs markedly among individuals. Given this ubiquitous variation among animals, a key question in my research is how do these 'many' become 'one'? In this talk, I will present new data from my research group examining this question both from a physiological and a behavioural standpoint. Our results show that both play an important role in shaping group structure as well as group composition. Furthermore, group membership feeds back to shape individual behaviour in unexpected ways.

## A4.2 PERSONALITY VARIATION DRIVES COLLECTIVE MOVEMENTS AND GROUP FUNCTIONING IN SCHOOLING FISH

📅 MONDAY 4 JULY, 2016 ⌚ 11:40

👤 JOLLE W JOLLES (MAX PLANCK INSTITUTE OF ORNITHOLOGY, GERMANY), NEELTJE BOOGERT (UNIVERSITY OF OXFORD, UNITED KINGDOM), ANDREA MANICA (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM)

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Collective behaviour can often emerge from individuals following simple interaction rules. The ubiquity of animal personalities raises the question if variation within groups is another layer that should be accounted for. Here by detailed individual-based tracking we investigated the effect of personality differences in the movements and foraging of freely-swimming stickleback shoals. Sociability was the key predictor of an individual's spatial positioning, network and leadership and drove the cohesion, alignment, and exploration of the group. Boldness was directly linked to an individual's propensity to search for and successfully secure food, and became a stronger predictor of individual and group movements with higher risk-

reward trade-offs. Ultimately, the interaction between the traits determined both individual foraging success and group foraging speed. These results show personality variation is a fundamental component of collective behaviour, with social attraction and risk-balancing being two fundamental but distinct behavioural axes.

## A4.3 ANT NUTRITION: FROM INDIVIDUAL NEEDS TO COLLECTIVE DECISION

📅 MONDAY 4 JULY, 2016 ⌚ 11:55

👤 AUDREY DUSSUTOUR (UNIVERSITE PAUL SABATIER, FRANCE)

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A fundamental question in nutritional biology is how distributed systems maintain an optimal supply of multiple nutrients essential for life and reproduction. We address this question using highly organised societies of ants. In the case of animals, the nutritional requirements of the cells within the body are coordinated by the brain in neural and chemical dialogue with sensory systems and peripheral organs. Being a social insect adds a level of complexity to nutritional regulatory strategies. Contrary to other animals, the food entering a social insect colony is assessed and collected by only a small number of workers. These foragers need to adjust their harvesting strategy to the internal demands for nutrients within the nest, where larvae and workers have different needs. So how do foragers reactions to food encountered outside the nest relate to the nutritional demands of the nest as a whole and themselves as individuals? Here, we show that foraging ants can solve nutritional challenges for the colony by making intricate adjustments to their feeding behaviour and nutrient processing, acting both as a collective mouth and gut.

## A4.4 MATERNAL EFFECTS ON SHOAL COHESION IN AN AFRICAN CICHLID (*DIMIDIOCHROMIS COMPRESSICEPS*)

📅 MONDAY 4 JULY, 2016 ⌚ 12:25

👤 TIFFANY ARMSTRONG (UNIVERSITY OF GLASGOW, UNITED KINGDOM), KEVIN PARSONS (UNIVERSITY OF GLASGOW, UNITED KINGDOM)

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Group living can increase survivorship of individuals and exists in a wider range of taxa and life stages. Maternal effects, the non-genetic

transfer of traits to from mother to offspring, are known to impact offspring growth, survival and in some cases social behaviour. It can be suggested that maternal effects should impact behaviours associated with group living, such as shoaling behaviours in fish. Fish species that maternally mouthbrood their young, such as many of the African cichlids, present a unique opportunity to examine these maternal effects. However, the effects this type of care could have on shoaling remains largely uncharacterised. Additionally, the longevity of maternal effects is uncertain and it is not clear if the effect on shoal cohesion will remain consistent. This study aimed to determine differences in shoal cohesion amongst groups of juveniles that were reared in an artificial environment and those reared in the natural maternal environment, as well as the effects over time, in a mouthbrooding African cichlid, *Dimidiochromis compressiceps*. To determine the effect of maternal care broods were split, with a portion being reared naturally by the mother and another artificially. To analyse the effects over time shoal cohesion in a novel environment was examined two weeks after juveniles were released from the mother (roughly one month post fertilization), five months post fertilization, and again at one year post fertilization. The effects of maternal care and family on shoal cohesion will be discussed, as well as how this changes over ontogeny.

#### A4.5 SHOALING REDUCES METABOLIC RATE IN A GREGARIOUS CORAL REEF FISH SPECIES

📅 MONDAY 4 JULY, 2016 ⌚ 12:40

👤 LAUREN E NADLER (JAMES COOK UNIVERSITY, AUSTRALIA), SHAUN S KILLEN (UNIVERSITY OF GLASGOW, UNITED KINGDOM), EVA C MCCLURE (JAMES COOK UNIVERSITY, AUSTRALIA), PHILIP L MUNDAY (JAMES COOK UNIVERSITY, AUSTRALIA), MARK I MCCORMICK (JAMES COOK UNIVERSITY, AUSTRALIA)

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Many animals live in groups due to the benefits that sociability can confer. For instance, group-living can reduce the energetic requirements needed to fuel a variety of processes, such as flight in birds, swimming in fish, web-building in spiders and thermoregulation in mice. Theory also suggests that group living may induce a 'calming effect' through a variety of mechanisms, reducing the physiological stress experienced by group members and, potentially, reducing individuals' total metabolic demand. However, this effect has proven difficult to quantify. In this study, we measured the impacts of shoaling on the metabolism and body condition of a gregarious coral reef fish species, the shoaling damselfish *Chromis viridis*. Using a novel respirometry methodology for a social species, we found that the presence of shoal-mate cues led to a significant reduction in the measured standard metabolic rate of individuals. Although all fish were fed a body-mass specific feeding regime, fish held in isolation exhibited a significant reduction in body condition following one week in treatment when compared to those held in shoals. Interestingly, individuals accustomed to the group holding treatment exhibited a stronger initial physiological reaction to stress than those acclimated to an isolated condition, potentially due to the stress of acute isolation and a lower threshold of threat at which they instigate a stress response. As environmental disturbances have the potential to induce social isolation, these results could have ecological consequences for gregarious species.

#### A4.6 POPULATION VARIATION IN MOUNTAIN ZEBRA SOCIAL NETWORKS: INDIVIDUALS, DEMOGRAPHY AND ECOLOGY IMPACT ON STRUCTURE

📅 MONDAY 4 JULY, 2016 ⌚ 13:55

👤 SUSANNE SHULTZ (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), JOHN JACKSON (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), JESSICA LEA (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

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Habitat quality has clear implications for population health, as sink populations are often unsustainable. However, the intermediate links between ecology and individual fitness, including population structure, behaviour and individual physiology are not well elucidated. Here we use conventional population and scale-free social network analyses to evaluate how habitat suitability impacts on variation in social network structure and demography across ten populations of the Cape mountain zebra (CMZ), *Equus zebra zebra*. Individual connectivity, the distribution of adult male ties, group size and the proportion of bachelor males within a population are all associated with the availability of palatable grass species and water. In good habitats, groups were larger, the population had a more balanced sex ratio, and individual connectivity and heterophily in the ties of adult males were higher. Populations in poor quality habitats also have poor female reproductive performance. We compare these results with those from semi-feral ponies, which suggest that individual network position is associated with reproductive success. Comparing social networks in populations with varying ecological pressures can highlight behavioural and demographic responses to ecological challenges and can highlight the consequences of network instability at the individual and population level.

#### A4.7 HETEROGENEITY IN ANIMAL COLLECTIVES

📅 MONDAY 4 JULY, 2016 ⌚ 14:35

👤 ANDREW J KING (SWANSEA UNIVERSITY, UNITED KINGDOM), CEDRIC SUEUR (UNIVERSITY OF STRASBOURG, FRANCE), INES FÜRTBAUER (SWANSEA UNIVERSITY, UNITED KINGDOM), GAELLE FEHLMANN (SWANSEA UNIVERSITY, UNITED KINGDOM), LEAH WILLIAMS (CHESTER ZOO, UNITED KINGDOM), CLAUDIA METTKE-HOFMAN (LIVERPOOL JOHN MOORES UNIVERSITY, UNITED KINGDOM), ANDREA MANICA (CAMBRIDGE UNIVERSITY, UNITED KINGDOM), DANIEL STRÖMBOM (LAFAYETTE COLLEGE, UNITED STATES), JUSTIN O'RIAIN (UNIVERSITY OF CAPE TOWN, SOUTH AFRICA), GUY COWLISHAW (INSTITUTE OF ZOOLOGY, UNITED KINGDOM)

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I will present collaborative research conducted on a variety of group-living fish, bird, and mammal systems in the wild and in the lab. I will show how novel technologies and analytical tools allow us access information about interactions at many spatial-temporal scales. Then, using real-life cases and theoretical models that deal with the causes and consequences of collective behaviour, I will highlight the importance and role of heterogeneity in animal collectives.

## A4.8 EXPOSURE TO NON-PREFERRED TEMPERATURES AS A COST OF SOCIABILITY IN INDIVIDUAL THREE-SPINED STICKLEBACKS

📅 MONDAY 4 JULY, 2016 ⌚ 14:50

👤 BEN COOPER (UNIVERSITY OF GLASGOW, UNITED KINGDOM), BART ADRIAENSSENS (UNIVERSITY OF GLASGOW, UNITED KINGDOM), SHAUN S KILLEN (UNIVERSITY OF GLASGOW, UNITED KINGDOM)

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Group behaviours occur in a number of taxa and provide a range of benefits, including increased safety from predators. There are also costs to group living, however, including increased competition and possibly reduced food-intake for some group members. An additional cost that has not been thoroughly studied is that individuals may sacrifice occupying their own optimal or preferred environmental conditions to experience the benefits of grouping.

This study investigated interactions between sociability and temperature preferences in individual three-spined stickleback (*Gasterosteus aculeatus*). 50 individuals from 10 families of captive-bred three-spined stickleback were first scored for individual temperature preference using a shuttle-tank setup in which they could behaviourally regulate their own temperature. Individuals were then allowed to choose between occupying two tank sections with a 3 degree temperature differential, with one tank section containing a shoal of siblings within a transparent cylinder. Each fish's position was tracked using a camera above the tank for 30 minutes. Trials were repeated with the shoal on the warmer side, then the cooler side, then without any temperature differential between the sides. Individual fish appear to forego exposure their own preferred temperature regime to be with a group of conspecifics. Importantly, however, the degree of thermal 'cost' experienced by each individual depends on their own preferred temperature as well as their baseline level of sociability. Results suggest that the costs and benefits of being within groups are not homogenous and that individual animals may have their own environmental thresholds which modulate their tendency to be social.

## A4.9 INTRASPECIFIC AGGRESSION DRIVES SYNCHRONOUS AIR-BREATHING AT HYPOXIA IN A CATFISH

📅 MONDAY 4 JULY, 2016 ⌚ 15:05

👤 SHAUN KILLEN (UNIVERSITY OF GLASGOW, UNITED KINGDOM), ANDREW ESBAUGH (UNIVERSITY OF TEXAS, UNITED STATES), TADEU RANTIN (FEDERAL UNIVERSITY OF SA7710 CARLOS, BRAZIL), DAVID MCKENZIE (UNIVERSITE769 MONTPELLIER, FRANCE)

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Air-breathing has evolved in multiple fish lineages and allows fish to access atmospheric oxygen. This is especially beneficial in hypoxic environments but comes with an increased risk of predation by aerial and terrestrial predators. To reduce individual predation risk, some species appear to synchronously air-breathe, whereby a group of fish surfaces simultaneously or within a short period of time. Given that individuals have variable physiological and behavioural thresholds

that trigger air-breathing, the mechanism by which individuals coordinate air-breathing is unknown. We examined this issue in groups of individual African sharp-tooth catfish at varying levels of dissolved oxygen in a laboratory arena. In agreement with previous reports we did observe synchronous air-breathing behaviour. There was high variability across groups in the total amount of surfacing behaviour displayed. The amount of air-breathing was strongly correlated with the amount of activity and particularly aggression occurring within each group. A similar trend was observed at the individual level: aggressive interactions were frequently followed by a series of air-breaths with subordinate individuals surfacing first. Synchronicity of air-breathing increased at hypoxia as did levels of aggression within groups. These results suggest that in sharp-tooth catfish, synchronous air-breathing is not cooperative but is instead driven by agonistic interactions that may expose subordinate individuals to a greater risk of predation. All fish were also measured for individual standard metabolic rate using bimodal respirometry. We are currently examining the extent to which air-breathing and aggression are related to metabolic rate and intrinsic tendency to breathe air when in isolation.

## A4.10 THE GOOD, THE BAD, AND THE UGLY: WHO IS REALLY BENEFITING FROM MOVING IN GROUPS?

📅 MONDAY 4 JULY, 2016 ⌚ 16:10

👤 STEVE J PORTUGAL (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM)

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Many species are highly gregarious and form large groups. These groups can serve multiple functions, such as enhancing predator detection and increasing foraging efficiency. Another key feature of why animals live in groups can be the benefits brought during collective locomotion. Travelling in groups can provide aero- or hydro-dynamic benefits, while groups of animals are known to home quicker, and more efficiently, than individuals travelling alone. However, such benefits are not always distributed equally throughout group members, and some individuals within a group will be benefitting disproportionately from travelling in groups, while others may be experiencing negative consequences. What determines how costs or benefits are distributed within a group is not fully understood, with both individual physiological and personality-based traits likely to play a role. This talk will present data looking at situations where benefits of travelling in groups are equally and non-equally distributed amongst members, and examine the underlying causes (physiological, behavioural, morphological) of this variation. Using a combination of biologging, respirometry and behavioural observations, case studies will focus on flocking in birds, the influence of dominance and social rank on movements in naked-mole rats, and how personality traits determine flock positioning in pigeons.

#### A4.11 TROUBLE IN PARADISE: WHAT DRIVES INDIVIDUAL VARIATION IN CLEANER WRASSE CHEATING BEHAVIOUR?

📅 MONDAY 4 JULY, 2016 ⌚ 16:40

👤 SANDRA A BINNING (UNIVERSITY OF NEUCHÂTEL, SWITZERLAND), OLIVIA REY (UNIVERSITY OF NEUCHÂTEL, SWITZERLAND), ALEXANDRA S GRUTTER (UNIVERSITY OF QUEENSLAND, AUSTRALIA), REDOUAN BSHARY (UNIVERSITY OF NEUCHÂTEL, SWITZERLAND)

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Cleaning mutualisms are textbook examples of inter-specific cooperation in socially complex marine systems: cleaners such as the wrasse, *Labroides dimidiatus*, eat ectoparasites from the surfaces of 'client' fish. But, there is often trouble in paradise. Cleaners can cheat by biting clients to eat mucus, which they prefer. To do so, cleaners can use tactical deception (providing massages to small clients) to lure large fish close before biting them. The extent to which cleaners cheat and whether they use deceptive strategies to maximize energy intake varies dramatically across individuals and habitats. However, the physiological and/or cognitive mechanisms and the role of the social environment in mediating these strategic decisions are unknown. We exogenously administered injections of corticosterone to induce physiological stress and incite cheating behaviour in cleaners from socially-complex or socially-simple habitats, and observed natural cleaning interactions over 45 minutes. We found that cleaners from socially-complex habitats, where intra-specific competition is high and reputation is essential for maximizing gains, use tactical deception to manipulate client partners when stressed: cortisol-injected cleaners provided more massages to small clients and bit large clients more often than saline-injected control fish. Conversely, cleaners from socially-simple habitats, where competition is lower and reputation is less important, did not use deceptive strategies, but instead increased their overall rates of cheating when injected with cortisol. These results demonstrate that a combination of social context, physiological state and learned decision rules mediate the switch from cooperation to cheating and tactical deception in cleaner wrasse.

#### A4.12 PROXIMATE MECHANISMS UNDERLYING VARIATION IN COOPERATION LEVELS BY THE CLEANER FISH LABROIDES DIMIDIATUS

📅 MONDAY 4 JULY, 2016 ⌚ 16:55

👤 DOMINIQUE G ROCHE (UNIVERSITY OF NEUCHÂTEL, SWITZERLAND), MAÏWENN JORNOD (UNIVERSITY OF NEUCHÂTEL, SWITZERLAND), ALEXANDRA GRUTTER (UNIVERSITY OF QUEENSLAND, AUSTRALIA), REDOUAN BSHARY (UNIVERSITY OF NEUCHÂTEL, SWITZERLAND)

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Game theoretic models help us understand how and when cooperation can evolve and persist. However, current models fall short of explaining the striking variation in cooperation levels that we observe in nature. For example, there is ample qualitative

evidence that supply and demand determine the exchange value of goods or services traded between cooperating partners ('biological market theory'). However, different species or individuals with similar partner choice options can be treated differently by the same partner. This is the case in the mutualism between the cleaner wrasse, *Labroides dimidiatus*, and its 'client' reef fishes. In this system, cleaners sometimes cheat and remove live tissue instead of ectoparasites. Client fishes differ not only in the number of cleaners they can visit, but also in a suite of other traits. To evaluate the relative importance of traits that might influence cleaning service quality, we examined 13 non-predatory client species and explored how different traits relate to cleaning service quality measured as the occurrence of cheating events in nature. Six variables were equally important in explaining variation in cooperation levels: interaction duration, client size, mucus amount and caloric content, gnathiid ectoparasite load, client turning rate and partner choice options. Partner choice is the cornerstone of biological market theory but was only one of many variables that regulate service quality in this marine mutualism. Our results suggest that future modelling efforts should integrate concepts such as temptation to cooperate/defect, partner choice options and punishment ability to better explain natural variation in cooperative behaviour.

#### A4.13 SOCIAL CONTEXT INFLUENCES RESPONSES TO HIGH TEMPERATURE CHALLENGE IN ISOGENIC LINES OF MANGROVE RIVULUS, *KRYPTOLEBIAS MARMORATUS*

📅 MONDAY 4 JULY, 2016 ⌚ 17:10

👤 SUZANNE CURRIE (MOUNT ALLISON UNIVERSITY, CANADA), LAURA STEEVES (MOUNT ALLISON UNIVERSITY, CANADA), KIRSTEN WEAGLE (MOUNT ALLISON UNIVERSITY, CANADA)

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Variation in physiological and behavioural traits within a species can be attributed to differences in genotype and/or differences in phenotype. Using isogenic lines of the self-fertilizing mangrove rivulus, we show that physiological responses to thermal challenge (e.g.  $CT_{max}$ , heat shock protein induction) are variable within one isogenic lineage. This suggests that it is largely phenotypic plasticity contributing to thermal tolerance with little influence of genotype. Moreover, when fish were subjected to high temperature challenge in pairs, the variation in thermal responses diminishes compared to when solitary fish are challenged. We further demonstrated that solitary fish had reduced thermal tolerance and a reduced heat shock response compared to paired fish from the same isogenic line. Thus, the social environment has an important role in how fish respond to ecologically relevant increases in water temperature, independent of genetic differences.

#### A4.14 AN INDIVIDUAL-BASED MODEL OF THE THREE-SPINED STICKLEBACK: INCORPORATING EFFECTS ON BREEDING BEHAVIOUR INTO THE ASSESSMENT OF ENDOCRINE DISRUPTING CHEMICALS

📅 TUESDAY 5 JULY, 2016      POSTER SESSION

👤 KATE S MINTRAM (EXETER UNIVERSITY, UNITED KINGDOM), PERNILLE THORBEC (SYNGENTA, UNITED KINGDOM), SAMUEL K MAYNARD (SYNGENTA, UNITED KINGDOM), A ROSS BROWN (EXETER UNIVERSITY, UNITED KINGDOM), CHARLES R TYLER (EXETER UNIVERSITY, UNITED KINGDOM)

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Population modelling is employed to extrapolate from individual effects (including behavioural effects) to population-level effects in the environmental risk assessment (ERA) of chemicals. In individual-based modelling, population dynamics and ecological processes such as density dependent competition emerge from interactions between individuals, including aspects of their behaviour. Reproduction in some fish species involves complex breeding behaviours that can be affected by chemical exposure but ERA does not incorporate aspects of behaviour into regulatory testing. In the three-spined stickleback (*Gasterosteus aculeatus*) nest building, courtship displays and parental care, may be disrupted by exposure to endocrine disrupting chemicals (EDCs), consequently potentially affecting population recruitment. Here, we present an individual-based model (IBM) for the three-spined stickleback with the purpose to simulate realistic scenarios for chronic exposure effects of EDCs. The three spined stickleback is widespread geographically, and potentially sensitive to chronic exposure to EDCs that mimic sex hormones given its complex breeding strategy, low fecundity and the provision of high level of parental care. Density dependent growth and individual breeding behaviours are key parameters within the model. The IBM has been structured using a series of sub-models, based on empirical data obtained from published literature. The poster will present a full description of the model with some preliminary testing, and illustrate its potential application within ERA.

#### A4.15 DOES FOOD DEPRIVATION AFFECT BEHAVIOUR IN JUVENILE EUROPEAN SEA BASS, *DICENTRARCHUS LABRAX*?

📅 TUESDAY 5 JULY, 2016      POSTER SESSION

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

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Food deprivation is a common environmental stressor, and because of their low metabolic rate and abilities to withstand starvation, ectotherms may survive well periods of food shortage that can last up to several months. All animals exhibit adaptive biochemical and physiological responses to the lack of food and these responses have been relatively well studied. Less studied, however, are the

behavioural responses employed to increase encounter rate with appropriate food items and/or to reduce energy requirement.

In a context where food resources are scarce, behavioural trade-offs between, for instance, boldness, shyness and sociability can play an important role in food seeking success and, therefore, in determining the survival of individuals. In the present experiment we investigated behavioural plasticity in fish by comparing boldness, swimming motivation and sociability in two populations of European sea bass (*Dicentrarchus labrax*) i.e., a population fed daily with maintenance ration and a population submitted to a 3-week fasting period. Individuals from each treatment were successively submitted to four experimental conditions, a new environment, novel object, hidden conspecific and a shelter. Expected results are that starvation will increase risk taking behaviour, increase interaction with a novel object, decrease social interaction and decrease time spent in the shelter.

#### A4.16 THE INTERPLAY BETWEEN SOCIAL HIERARCHY FORMATION AND METABOLIC TRAITS IN THE COMMON MINNOW

📅 TUESDAY 5 JULY, 2016      POSTER SESSION

👤 BROOKE ALLAN (UNIVERSITY OF GLASGOW, UNITED KINGDOM), SHAUN KILLEN (UNIVERSITY OF GLASGOW, UNITED KINGDOM)

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Metabolic rate shows wide individual variation within species and can affect social interactions within animal groups. However, it is also possible that social status within a group can feedback to affect individual metabolic traits due to social stress or differing levels of food intake among individuals. We examined these issues in the common minnow by measuring metabolic rate and aerobic scope before and after the formation of stable social hierarchies. Shoals of four individuals each were examined for food intake, aggressive interactions, and spatial positioning within their respective holding tanks over a period of 10 weeks. Metabolic rate and aerobic scope for each individual were measured using intermittent flow respirometry before and after hierarchy formation. Levels of aggression varied widely among individuals but also among shoals, both before and after feeding. Certain individuals were consistently more aggressive within shoals and their overall level of aggression appeared to affect the behaviour of the entire group. Spatial positioning was not related to social status among individuals. Changes in metabolic traits over the course of the study will be analysed in relation to the social status achieved by each individual. Results will provide insight into the cause and effect relationship between metabolic traits and social behaviours.

# A5 OSMOREGULATION AT THE EXTREMES: WATER AND ION BALANCE AT ENVIRONMENTAL HIGHS AND LOWS

**ORGANISED BY:** DR PEDRO GUERREIRO (UNIVERSITY OF ALGARVE, PORTUGAL), DR JONATHAN WILSON (UNIVERSITY OF PORTO, PORTUGAL) AND DR JUAN FUENTES (UNIVERSITY OF ALGARVE, PORTUGAL)

**SESSION SPONSORED BY:** LOLIGO SYSTEMS

## A5.1 BIOCHEMICAL ADAPTION AND MOLECULAR EVOLUTION OF FISH IN RESPONSE TO SALINITY STRESS

📅 THURSDAY 7 JULY, 2016 ⌚ 09:00

👤 DIETMAR KÜLTZ (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES)

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Many orders of fish contain species that have evolved the capacity to live at salinity extremes as high as 130 g salt per kg water and fluctuating as much as from fresh water to almost 4x seawater. For example, black-chinned tilapia inhabit fresh water streams and lakes as well as the Saloum estuary/ delta (Senegal) where salinity extremes can reach 130 g/kg. What makes these extremely euryhaline species of fish so resilient towards salinity stress and what biochemical mechanisms do they utilize to maintain proper cell and organ function? A systems biology approach based primarily on quantitative proteomics is presented that provides in-depth mechanistic insight into biochemical adaptations of euryhaline tilapia and sticklebacks to salinity extremes. Key nodes in the pertinent biochemical networks are identified based on network modeling/visualization. Casual relationships between these key nodes and salinity tolerance are established using genetic engineering of cell lines derived from these species. The results of these studies support the central role of organic osmolytes in salinity stress responses of bony fish. Despite bony fish being osmoregulators they are still subject to significant changes in plasma osmolality during salinity stress, which necessitates the regulation of organic osmolytes. In addition, novel proteins representing key nodes involved in osmotic stress signaling of euryhaline teleosts have emerged from these studies and their roles in corresponding biochemical/signaling pathways are discussed.

## A5.2 ARE THERE ANY EXTRABRANCHIAL SALT SECRETING ORGANS IN THE TELEOSTS?

📅 THURSDAY 7 JULY, 2016 ⌚ 09:40

👤 SALMAN MALAKPOOR (CIIMAR CENTRO INTERDISCIPLINAR DE INVESTIGAÇÃO MARINHA E AMBIENTAL, PORTUGAL), DR. JONATHAN MARK WILSON (CIIMAR CENTRO INTERDISCIPLINAR DE INVESTIGAÇÃO MARINHA E AMBIENTAL, PORTUGAL)

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The euryhaline Plotosidae catfish *Plotosus lineatus* is unique amongst the teleosts in that it possesses a specialized salt secreting organ, the dendritic organ (DO) whereas other marine teleosts rely on their gill ionocytes. In the present study, we investigated the effects of salinity [brackish water (BW) 3‰, seawater (SW-control) 34‰, hypersaline water (HSW) 60‰] on  $\text{Na}^+/\text{K}^+$ -ATPase (NKA) and  $\text{Na}^+:\text{K}^+:\text{2Cl}^-$  cotransporter (NKCC1) expression to elucidate the importance of the DO in ion-regulation, and DO ligation to characterize the compensatory responses of the gill and kidney. Our results show that DO NKA activity was significantly higher than in gill, kidney or intestine at all salinities; however, NKA activity only increased with HSW in kidney, and intestine posterior but not in either gill or DO. BW acclimation resulted in lower NKA activity in gill, kidney and DO. NKCC1 expression was high in DO but not detectable by immunoblot or immunohistochemistry in gill, kidney, or intestine. DO size increased with salinity and strong NKA/NKCC1 immunolocalization was observed which was absent in gill ionocytes. This latter observation contrasts with practically all other marine teleosts in which gill ionocytes are central to excess ion excretion. In SW, DO ligation did not alter gill or kidney NKA expression but significant changes were seen in the intestine. In BW, DO ligation only lowered gill NKA activity. In summary NKA activity is high in DO and co-expressed with NKCC1 which indicates a conservation of rather similar mechanisms of ion transporting in the convergent evolution of salt secreting organs in vertebrates.

### A5.3 TOLERANCE AND RESPONSES OF THE FRESHWATER SNAIL (*THEODOXUS FLUVIATILIS*) TO INCREASING SALINITIES

THURSDAY 7 JULY, 2016 09:55

AMANDA A. WIESENTHAL (ERNST MORITZ ARNDT-UNIVERSITY GREIFSWALD, GERMANY), DANA GOTTSCHLING (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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The snail *Theodoxus fluviatilis* (Gastropoda: Neritidae) is found in both brackish and freshwater habitats in northern Germany and has formed regional subgroups. These two lineages are closely related, and individuals of both are neither clearly distinguishable by shell size, shell patterning - due to high variability within each lineage - nor by mitochondrial RNA markers. Despite the high degree of similarity, they differ in their tolerance to changing salinities. Animals of the brackish water lineage struggle when transferred to freshwater and freshwater animals die upon transfer to high salinities (21‰). The low survival of freshwater animals may be explained by a less well developed ability to accumulate free amino acids in their foot muscle as a means of cell volume regulation. This diverging ability between the lineages is associated with characteristic differences in protein expression that show both, lineage-specific (genetic) and environmentally induced (plasticity) expression patterns. To elucidate any responses of these animals to changing salinities, including tolerance limits and experimentally achievable range shifts in reaction norms of freshwater animals to hypertonic environments, individuals were collected at three freshwater sites and exposed to a 22-day transfer and acclimation regime. Survival rate, the amount of accumulated free amino acids as well as protein patterns were recorded and analysed for each individual and each treatment. A control group and 3 treatments of either slow acclimatising or radical transfer to higher salinities were compared. The results showed that range shifts in reaction norms can be achieved by stepwise acclimation of freshwater animals to higher salinities.

### A5.4 OSMOREGULATORY DIVERGENCE IN A LANDLOCKED POPULATION OF *GALAXIAS MACULATUS* (JENYNS, 1848) IN THE ANDES

THURSDAY 7 JULY, 2016 10:10

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The amphidromous teleost fish, *Galaxias maculatus*, is found mainly migrating between estuaries and rivers, but some landlocked populations have been described in the Andes. In the present study we use mtDNA sequences to reconstruct the historical scenario of lake colonization and evaluated the potential osmoregulatory shift associated to changes in habitat and life cycle between amphidromous and landlocked populations. Standard diversity indices including the average number of nucleotide differences ( $\Pi$ ) and the haplotype diversity index (H) indicated that both populations were, as expected, genetically distinctive, being the landlocked population less diverse than the diadromous one. Similarly, pairwise  $G_{ST}$  and  $N_{ST}$  comparison detected statistically significant differences between both populations, while genealogy of haplotypes evidenced a recent founder effect from the diadromous stock, followed by an expansion process in the lake. To test for physiological differences, individuals of both populations were challenged, after progressive acclimation, with a range of salinities from 0 to 30 ppt for 8 days. The results showed that the landlocked population had a surprisingly wider salinity tolerance than diadromous fish. The activity of ATPase, including  $Na^+/K^+$ -ATPase, and  $H^+$ -ATPase was measured in gills and intestine. Activity differences were detected between the populations at the lowest salinities. These results clearly demonstrate the striking adaptive changes of *G. maculatus* osmoregulatory system, especially at hypotonic environments, associated to a drastic shift in habitat and life cycle in a few thousand years.

## A5.5 STRONG ION REGULATORY ABILITIES ENABLE THE CRAB *XENOGRAPSUS TESTUDINATUS* TO INHABIT THE WORLD'S MOST ACIDIC VENTS SYSTEMS

THURSDAY 7 JULY, 2016 10:55

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The under water volcano of Turtle island (Kuei-shan dao) off the coast of Taiwan has been described as one of the most acidic marine vent systems in the world, discharging water with a high content of elemental sulfur particles, temperatures of 76-116°C and a minimum pH of 1.25. This challenging hydrothermal vent habitat is inhabited by *Xenograpsus testudinatus*, a crab species that is endemic to shallow-water (>200m) vent systems. To survive in this highly acidified environment, this species has evolved substantial acid-base regulatory mechanisms. Within few hours *X. testudinatus* restores extracellular pH (pHe) in response to environmental acidification of pH 6.5 (1.78 kPa pCO<sub>2</sub>) accompanied by an increase in extracellular HCO<sub>3</sub><sup>-</sup> levels from 8.78 mM to 30.74 mM. The major branchial ion pumps including Na<sup>+</sup>/K<sup>+</sup>-ATPase (NKA) and V-type H<sup>+</sup>-ATPase (VHA), showed dynamic increases in response to acidified conditions on the mRNA, protein and activity level. A high timely resolution of the compensation reaction demonstrates a time lag between mRNA expression and synthesis of the enzymes by 2h and 6h for the NKA and VHA, respectively. Immunohistochemical analyses demonstrate the presence of NKA in basolateral membranes whereas the VHA has a predominantly cytoplasmic localization in branchial epithelia. This localization of the VHA in vesicles and its strong up regulation during environmental acidification suggest pH regulatory mechanisms via vesicular pathways. Our results demonstrate that strong acid-base regulatory abilities are probably a key feature of this crab species to successfully inhabit one of the world's most acidic marine environments.

## A5.6 INFLUENCE OF REDUCED SALINITY AND ELEVATED CO<sub>2</sub> ON OSMOREGULATION IN TWO SPECIES OF CRABS: AN OSMOREGULATOR VS AN OSMOCONFORMER

THURSDAY 7 JULY, 2016 11:25

NIA M WHITELEY (BANGOR UNIVERSITY, UNITED KINGDOM), COLEEN C SUCKLING (BANGOR UNIVERSITY, UNITED KINGDOM), BEN J CIOTTI (PLYMOUTH UNIVERSITY, UNITED KINGDOM), JAMES BROWN (BANGOR UNIVERSITY, UNITED KINGDOM), IAN D MCCARTHY (BANGOR UNIVERSITY, UNITED KINGDOM), LUIS GIMENEZ (BANGOR UNIVERSITY, UNITED KINGDOM), CHRIS HAUTON (UNIVERSITY OF SOUTHAMPTON, UNITED KINGDOM)

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The osmotic responses of two species of crabs with differing abilities to compensate for environmental change were investigated as part of a larger study into the metabolic restrictions associated with physiological adjustments. The shore crab, *Carcinus maenas*, a weak osmoregulator and the edible crab, *Cancer pagurus*, an osmoconformer were collected from the shore and exposed to dilute seawater (S=25) and a reduction in seawater pH (7.6-7.7) for up to 12 months. Changes in haemolymph osmolality and acid-base status were determined at various timepoints to assess compensatory capacities. Branchial Na<sup>+</sup>/K<sup>+</sup> ATPase activities and gene expression levels for specific branchial ion exchangers and key enzymes were also determined to examine the underlying mechanisms. *C. maenas* was able to maintain haemolymph acid-base status despite reductions in salinity and seawater pH, but haemolymph pH decreased in *C. pagurus* over time. Haemolymph osmolality decreased in both species on exposure to dilute seawater, but mean values remained 50 to 170 mOsmol<sup>-1</sup> higher than seawater values in *C. maenas* and only 30-80 mOsmol<sup>-1</sup> higher in *C. pagurus*. Seawater dilution resulted in a significant increase in branchial Na<sup>+</sup>/K<sup>+</sup> ATPase activities in *C. maenas* and had significant effects on gene expression levels for Na<sup>+</sup>/K<sup>+</sup> ATPase; Na<sup>+</sup>/H<sup>+</sup>; anion exchanger, V-type H<sup>+</sup> ATPase and cytoplasmic carbonic anhydrase. None of these changes were observed in *C. pagurus* but crabs still survived the treatments for up to 9 months. Mechanisms, and therefore, metabolic consequences and survival prospects appear to differ between the species.

## A5.7 FISHES OF THE RIO NEGRO: THE CHALLENGES OF LEAVING IN AN EXTREME ENVIRONMENT

THURSDAY 7 JULY, 2016 13:50

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The Rio Negro drains into the Amazon River and is the largest blackwater river in the world. Its water is remarkably poor in ions, with conductivity below 10  $\mu$ S, extremely acidic, with pH ranging from 2.9 to 5.0, and rich in dissolved organic carbon (DOC), which levels of up to 30 mg/L vary season and regionally. Near 300,000 Km<sup>2</sup> of the Rio Negro basin is annually flooded, which includes one of the largest river archipelago, the Anavilhanas. The flooded forest (known as igapó) is visited by many fish species searching for food. These species are adapted to hypoxia and very acidic ion-poor waters. The Rio Negro is the house of more than 1,200 fish species belonging to different groups, including stingrays, ornamental fishes, such as the cardinal tetras, and the largest freshwater air-breathing fish pirarucu. How these fishes can thrive these acidic ion-poor waters, continually defy fish biologists. Over the last few years, we learned that behavioral, physiological and biochemical characteristics, that require regulation of gene transcription, allow these fishes to thrive the extreme environments of the Rio Negro. Also, it is possible that the presence of high levels of DOC, which quality varies geographically and along the year, also plays a major role in protecting the animals. Undoubtedly, the fish of the Rio Negro constitutes a singular group of animals facing extreme osmoregulation challenges. (INCT ADAPTA-CNPq/FAPEAM).

## A5.8 IDENTIFICATION OF BRANCHIAL ACID-BASE EXCRETING IONOCYTES IN A STENOHALINE FRESHWATER ELASMOBRANCH (POTAMOTRYGON SPP.)

THURSDAY 7 JULY, 2016 14:30

JONATHAN M WILSON (WILFRID LAURIER UNIVERSITY, CANADA), MARK W ROSSI (WILFRID LAURIER UNIVERSITY, CANADA), CHRIS M. WOOD (UBC, CANADA), ADALBERTO L. VAL (INPA, BRAZIL)

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The branchial iono acid-base regulatory strategies of the Amazonian freshwater rays (genus: Potamotrygon) that have adapted to extremely ion poor water were investigated using immunohistochemistry. In elasmobranchs, branchial ionocytes have been shown to be important to acid-base regulation as well as ion uptake in freshwater euryhaline species. The Potamotrygon have been isolated in freshwater for well over 10 million years and are stenohaline. They have also yet to have their mechanisms of branchial ionoregulation investigated. Branchial ionocytes were characterized as either (A) acid secreting or (B) base secreting types. A-type ionocytes were identified as clusters of Na<sup>+</sup>/K<sup>+</sup> ATPase immunoreactive cells with apical Na<sup>+</sup>/H<sup>+</sup> exchanger 3-like immunoreactivity. These clusters of cells were only found in the filament epithelium towards the afferent side. B-type ionocytes were found scattered throughout the lamellar and filament epithelia and were characterized by strong cytosolic-basolateral vacuolar-type H<sup>+</sup>

-ATPase immunoreactivity and apical pendrin Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup>-exchanger staining. The responsiveness of A and B-type ionocytes to acid-base disturbances will be discussed in the paper.

## A5.9 WATER IMMERSION-INDUCED HYPOXIA DEMANDS SHIFT IN THE ION TRANSPORTER FUNCTION IN THE BRAIN OF AIR-BREATHING FISH (ANABAS TESTUDINEUS BLOCH): EVIDENCE FOR INTEGRATIVE ROLES OF ION-MOTIVE ATPASES

THURSDAY 7 JULY, 2016 14:45

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Bony fishes possess an array of ion transporters that efficiently maintain the cellular and systemic ion homeostasis. It is hypothesized that hypoxic challenge may modify the pattern of ion transporter functions in fish brain. We, therefore, investigated the pattern of ion transporter function in the brain of hypoxic air-breathing fish (*Anabas testudineus* Bloch) at varied time intervals. Water immersion was practiced to induce hypoxic stress in fish. Analysis of the kinetic pattern of ion-motive ATPases viz Na<sup>+</sup>/K<sup>+</sup>-ATPase, H<sup>+</sup>/K<sup>+</sup>-ATPase and Na<sup>+</sup>/NH<sub>4</sub><sup>+</sup>-ATPase revealed that these transporters are actively involved in the exchange of Na<sup>+</sup>, K<sup>+</sup>, H<sup>+</sup> and NH<sub>4</sub><sup>+</sup> across neural membrane. mRNA expression of nka $\alpha$  subunit isoforms; nka $\alpha$ 1a, nka $\alpha$ 1b and nka $\alpha$ 1c in the varied regions of brain also showed a modified response to experimental hypoxia. It appears that the differential expression of nka $\alpha$  isoforms and its temporal and spatial distribution implies that switching of nka $\alpha$ -subunit isoform diversity exists as part of brain response to hypoxic stress. Collectively, the data indicate that hypoxia can demand a modified differential/integrative pattern of ion transporter function in the brain of air-breathing fish.

### A5.10 POTASSIUM AND ACID-BASE REGULATION IN THE LUNGFISH (PROTOPTERUS ANNECTENS): A ROLE FOR THE NON-GASTRIC $H^+/K^+$ -ATPASE?

📅 THURSDAY 7 JULY, 2016 ⌚ 15:00

👤 JUSTINE DOHERTY (WILFRID LAURIER UNIVERSITY, CANADA), LUÍS FILIPE C CASTRO (CIIMAR, PORTUGAL), MICHAEL P WILKIE (WILFRID LAURIER UNIVERSITY, CANADA), YUEN KWONG IP (NATIONAL UNIVERSITY OF SINGAPORE, SINGAPORE), JONATHAN M WILSON (WILFRID LAURIER UNIVERSITY, CANADA)

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The lungfish *Protopterus annectens* is capable of living under some extreme conditions which include estivation during periods of drought. In this study the hydrogen/potassium  $H^+/K^+$ -ATPase (HKA) and its hypothesized role(s) in acid-base and potassium ( $K^+$ ) regulation are investigated. There are two types of HKA in vertebrates: the gastric and non-gastric forms. This work is focused on the non-gastric (ng) HKA form that is composed of a unique  $\alpha$  subunit (HK $\alpha$ 2, *atp12a*) while the gastric HKA (HK $\alpha$ 1, *atp4a*) is not present in stomach-less lungfish. We have cloned and sequenced the full length coding region for *P. annectens atp12a*. Using a RT-PCR based approach we have determined that gill and kidney, which are the two main ionoregulatory organs in fishes, have the highest mRNA expression levels in an organ panel. An anti-peptide antibody against lungfish HK $\alpha$ 2 has been developed and validated for immunoblotting and we have corroborated that gill and kidney have high protein expression levels as well. Finally, we have developed a non-radioactive method for measuring uptake rates using rubidium ( $Rb^+$ ) as a surrogate for potassium and have measured rubidium flux rates that are in agreement with predicted values. Together, these data indicate that the non-gastric  $H^+/K^+$ -ATPase is expressed in key ionoregulatory organs and that  $Rb^+$  uptake occurs. However, short term acid-base disturbances (metabolic acidosis or alkalosis), and fasting (14 days) did not alter either  $Rb^+$  fluxes or gill HK $\alpha$ 2 protein levels. The significance of our findings will be discussed.

### A5.11 NOVEL WATER TRANSPORT IN MARINE TELEOSTS - CARBONATE PRECIPITATION CHALLENGES DOGMA BUT CAN'T TAKE THE PRESSURE (OR COLD)

📅 THURSDAY 7 JULY, 2016 ⌚ 15:45

👤 ROD W WILSON (UNIVERSITY OF EXETER, UNITED KINGDOM), JON M WHITTAMORE (UNIVERSITY OF FLORIDA, UNITED STATES), ERIN R REARDON (UNIVERSITY OF EXETER, UNITED KINGDOM), MAURICIO A URBINA (UNIVERSIDAD DE CONCEPCIÓN, CHILE), JONATHAN M WILSON (WILFRID LAURIER UNIVERSITY, CANADA)

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Animals are ~70% water and epithelial water fluxes are vast, yet the mechanisms of fluid transport remain controversial. It usually accepted that this first requires net solute transport to secondarily drive net fluid transport in the same direction by osmosis, i.e. solute-coupled fluid transport. However, high rates of bicarbonate secretion into the intestine of marine teleosts (the most speciose vertebrates) cause the alkaline precipitation of carbonates, reducing osmolality and driving net water absorption. This challenges a central dogma of water transport, not relying on solute transport in the same direction as water. Here we show evidence from a range of approaches including 1) in vitro experiments mimicking intestinal chemistry changes during  $CaCO_3$  precipitation, 2) ex vivo experiments (intestinal gut sacs) comparing NaCl and water fluxes under modified osmotic gradients, 3) meta-analysis of 693 fish osmoregulation studies across the global range of marine temperatures (0-30°C), 4) in vivo experiments exploring how low temperature and high pressure (that enhance carbonate solubility and limit precipitation) affect osmoregulation (blood chemistry, drinking and fluid absorption), and 5) comparative field studies at extremes (Antarctic nototheniids, deep-sea fish at 2,500-3,500 m, and mesopelagics migrating between 1000 and 55 m depth). All these approaches support the model of substantial water transport being driven by carbonate precipitation in the intestine of marine teleost fish. Furthermore, this physiological mechanism of water transport helps explain the impact of environmentally relevant low temperatures and high pressure (i.e. depth) on the effectiveness of osmoregulation in marine fish.

## A5.12 IDENTIFICATION OF RESPONSIVENESS ACID-BASE MARKERS IN EMBRYONIC TELEOSTS UNDER CO<sub>2</sub>-DRIVEN SEAWATER ACIDIFICATION

THURSDAY 7 JULY, 2016 16:15

YUNG-CHE TSENG (NATIONAL TAIWAN NORMAL UNIVERSITY, TAIWAN), MARIAN Y. HU (CHRISTIAN-ALBRECHTS UNIVERSITY KIEL, GERMANY), PEI-CHEN HUANG (NATIONAL TAIWAN NORMAL UNIVERSITY, TAIWAN), TZU-YEN LIU (NATIONAL TAIWAN NORMAL UNIVERSITY, TAIWAN)

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Elevated atmospheric CO<sub>2</sub> has lately caused considerable effects on marine ecosystems and leads to shifts of pH value in ocean. For those athletic marine animals, teleosts are well known as strong acid-base regulators that are capable of accumulating HCO<sub>3</sub><sup>-</sup> in body fluids to fully compensate for CO<sub>2</sub> induced acid-base disturbances. In this study, we monitored developing appearances and transcripts expression profiling involved in acid-base regulation to study the effects of CO<sub>2</sub>-driven seawater acidification on two euryhaline medaka sister-species, the freshwater medaka *Oryzias latipes* (Japanese medaka), and the marine medaka *Oryzias melastigma* (Indian medaka). Our results demonstrate that, on one hand, *O. latipes* embryos respond with delayed development during the time window of 2-5 dpf when exposed to a seawater pCO<sub>2</sub> of 0.12 and 0.42 kPa. On the other hand, the growth bottleneck appearance in marine species *O. melastigma* is not significant as *O. latipes*. Moreover, transcripts levels of anion exchanger 1b (AE1a), Na<sup>+</sup>/HCO<sub>3</sub><sup>-</sup>-exchanger a (NBCa) and carbonic anhydrase 15 (CA15) were found to be both up-regulated in these two species for controlling of bicarbonate homeostasis during ambient hypercapnia. And the proton secretion pathway is as well achieved via apical Na<sup>+</sup>/H<sup>+</sup>-exchanger (NHE) in epithelium as this SLC9 protein member is thermodynamically favorable due to high external [Na<sup>+</sup>] compared to low intracellular [Na<sup>+</sup>] in the marine environment. Consequently, the present study elucidates that HCO<sub>3</sub><sup>-</sup> and pH modulations could provide a homeostatic basis in early embryonic teleosts to cope with CO<sub>2</sub>-driven seawater acidification.

## A5.13 FASTING IN THE 'BIG SPENDER' LAKE MAGADI TILAPIA AFFECTS IONOREGULATION RATHER THAN ENERGY BUDGET

THURSDAY 7 JULY, 2016 16:30

GUDRUN DE BOECK (UNIVERSITY OF ANTWERP, BELGIUM), CHRIS M WOOD (UNIVERSITY OF BRITISH COLUMBIA, CANADA), KEVIN M BRIX (ECOTOX MIAMI, CANADA), AMIT K SINHA (UNIVERSITY OF ANTWERP, BELGIUM), ORA E JOHANNSSON (UNIVERSITY OF BRITISH COLUMBIA, CANADA), ADALTO BIANCHINI (UNIVERSIDADE FEDERAL DO RIO GRANDE, BRAZIL), LUCAS F BIANCHINI (UNIVERSIDADE FEDERAL DO RIO GRANDE, BRAZIL), JOHN N MAINA (UNIVERSITY OF JOHANNESBURG, SOUTH AFRICA), GERALDINE D KAVEMBE (SOUTH EASTERN KENYA UNIVERSITY, KENYA), MICHAEL B PAPA (UNIVERSITY OF NAIROBI, KENYA), KISIPAN M LETURA (EGERTON UNIVERSITY, KENYA), RODI O OJOO (UNIVERSITY OF NAIROBI, KENYA)

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Lake Magadi, Kenya is one of the most extreme aquatic environments on earth (pH~10, anoxic to hyperoxic, high temperatures). *Alcolapia grahami*, the only fish surviving in the lake is the only known 100% ureotelic teleost and it shows among the highest aerobic metabolisms seen in fish. For food, they largely depend on available cyanobacteria. This food limitation, combined with their high metabolism, often gives them a skinny appearance. During a 5-day starvation period, metabolic rates actually increased, but urea excretion remained stable leading to a lower nitrogen quotient as expected when devoid of their N-rich food source. Tissue protein levels tended to decrease after a 5-day fast. Fish relied heavily on carbohydrates with lowered plasma glucose, lactate and muscle glycogen. However, fish were not able to maintain ion homeostasis with reduced plasma osmolarity and Na (but not Cl) levels, despite increased expression levels of gill, gut and kidney Na/KATPase. In contrast, expression of gill and gut urea transporters reduced, as did gill Rhesus glycoprotein Rhbg and Rhcg. Even though Lake Magadi tilapia do not excrete ammonia, it still plays a vital role in protein metabolism. The reduction in gill glutamine synthetase concomitant with the reduction in Rh glycoprotein indicates reduced nitrogen metabolism. Gill pavement cells showed a reduced surface area as they lost the microridges on their surface. As suggested by Wood and co-workers in 2002 (*Physiol. Biochem. Zool.* 75) iono- and acid-base regulation demands a substantial amount of energy in these fish, and was compromised during food deprivation.

### A5.14 OSMOREGULATORY AND METABOLIC RESPONSES IN THE ANTARCTIC NOTOTHENIA ROSSII EXPOSED TO ALTERED SALINITY AND THERMAL REGIMES

THURSDAY 7 JULY, 2016 16:45

PEDRO M GUERREIRO (CENTRE FOR MARINE SCIENCES, PORTUGAL), ALEXANDRA ALVES (CENTRE FOR MARINE SCIENCES, PORTUGAL), BRUNO LOURO (CENTRE FOR MARINE SCIENCES, PORTUGAL), ELSA COUTO (CENTRE FOR MARINE SCIENCES, PORTUGAL), JONATHAN M WILSON (CIIMAR, PORTUGAL), ADELINO VM CANARIO (CENTRE FOR MARINE SCIENCES, PORTUGAL)

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The Antarctic Ocean is one of the most extreme marine environments, with temperatures reaching -1.9°C. Antarctic fishes evolved in a stable thermohaline conditions for roughly 30 million years, currently displaying a number of structural and functional features that favour adaptation. Recent climate changes have contributed to rises in water temperature and forecast models indicate the rate of such changes will increase in coastal regions of maritime Antarctica, leading to ice melting and freshening of shallow waters in enclosed areas. Fish, *Notothenia rossii* and *Notothenia coriiceps*, collected around Rothera (UK), Arctowski (PL) and Great Wall (CN) stations in Adelaide and King George Islands were transferred to experimental tanks and acclimated from natural temperatures (0-2°C) to 4-8°C using thermostat-controlled heaters, and from 32‰ to 20-10‰ by addition of freshwater to recirculating tanks, over a period of up to 10 days. Plasma and urine electrolytes and renal and branchial  $\text{Na}^+/\text{K}^+$ -ATPase were determined, and tissues were collected for immunohistochemistry and gene expression. Altered conditions had no effect in immediate mortality, but reduced overall activity and startling time. Cortisol and gene expression of metabolic/osmotic-related proteins were modified after heat and salinity shock. Temperature induced dependent decrease in plasma osmolality, increasing the osmotic gradient between extracellular fluid and seawater and resulting in increased branchial/renal  $\text{Na}^+/\text{K}^+$ -ATPase activity. Low salinity reduced both plasma and urine osmolality, with fish showing a marked increase in urine production, and significant changes in urine ionic composition and urine/plasma ratio. Results also indicate these fish possess important mechanisms for water elimination despite their aglomerular kidneys.

### A5.15 PHYSIOLOGICAL AND CELLULAR MECHANISMS UNDERLYING SALINE TO FRESHWATER INVASIONS BY THE COPEPOD *EURYTEMORA AFFINIS*

WEDNESDAY 6 JULY, 2016 POSTER SESSION

GUY CHARMANTIER (UNIVERSITE DE MONTPELLIER, FRANCE), LUCIE GERBER (UNIVERSITE DE MONTPELLIER, FRANCE), KELSEY E. JOHNSON (UNIVERSITY OF WISCONSIN, UNITED STATES), LUCILE PERREAU (UNIVERSITE DE MONTPELLIER, FRANCE), MARIJAN POSAVI (UNIVERSITY OF WISCONSIN, UNITED STATES), EVA BLONDEAU-BIDET (UNIVERSITE DE MONTPELLIER, FRANCE), EVELYSE GROUSSET (UNIVERSITE DE MONTPELLIER, FRANCE), MIREILLE CHARMANTIER-DAURES (UNIVERSITE DE MONTPELLIER, FRANCE), CATHERINE LORIN-NEBEL (UNIVERSITE DE MONTPELLIER, FRANCE), CAROL E. LEE (UNIVERSITY OF WISCONSIN, UNITED STATES)

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Colonizations from marine to freshwater (FW) environments constitute dramatic evolutionary transitions in the history of life, and pose great osmoregulatory challenges for organisms. The copepod *Eurytemora affinis*, which lacks gills, has recently invaded FW habitats multiple times independently in North America. We measured hemolymph osmolality for ancestral saline and FW invading populations reared at different salinities (0.2-25 PSU). We found evolution of increased hemolymph osmolality (by 16-31%) at lower salinities in FW populations relative to their saline ancestors. Using silver staining, immunolocalization of  $\text{Na}^+/\text{K}^+$ -ATPase (NKA) and V-H<sup>+</sup>-ATPase (VHA), and TEM, we localized ion transport within the maxillary glands and in novel osmoregulatory structures containing ionocytes, which we named the 'Crusalis organs' at the five pairs of swimming legs. Semi-quantification of *in situ* expression of NKA and VHA established the predominance of legs 3 and 4 in ion transport in both saline and FW populations. Increases in VHA expression in legs 3 and 4 of the FW population (in FW) relative to the saline population (at 15 PSU) arose from an increase in abundance of VHA per cell, rather than increased number of ionocytes, suggesting a simple mechanism for increasing ion uptake in FW. In contrast, the decline in NKA expression in the FW population arose from a decrease in ionocyte area in legs 4, likely resulting from decreases in number or size of ionocytes. Such results provide insights into mechanisms of ionic regulation for this species, with added insights into evolutionary mechanisms underlying physiological adaptation during habitat invasions.

## A5.16 DISCONTINUOUS GAS EXCHANGE DOES NOT CONTRIBUTE TO INCREASED RESISTANCE TO DESICCATION IN LABORATORY-SELECTED POPULATIONS OF THE MIGRATORY LOCUST

WEDNESDAY 6 JULY, 2016 POSTER SESSION

ERAN GEFEN (UNIVERSITY OF HAIFA- ORANIM, ISRAEL), STAV TALAL (TEL AVIV UNIVERSITY, ISRAEL), AMIR AYALI (TEL AVIV UNIVERSITY, ISRAEL)

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A leading hypothesis for the evolution of discontinuous gas exchange cycles (DGCs) in insects is the hygrich hypothesis, which posits that DGCs serve to reduce respiratory water loss. In this study we tested predictions of this hypothesis, by using an experimental evolution approach. We compared populations of the migratory locust (*Locusta migratoria*), after 10 generations of selection for desiccation-resistance, with control ones. Survival time, at 30°C with access to fresh food denied, was 36% longer in the selected compared with control locusts (8.3±0.4d and 6.1±0.3d, respectively). Significantly higher body water content when hydrated, and lower evaporative water loss rates were recorded in the selected locusts. They also exhibited significantly longer DGCs than controls, resulting from longer interburst, but not burst, durations. However, in contrast with predictions of the hygrich hypothesis, populations did not vary in DGC prevalence. Additionally, evolved changes in DGC properties in the selected locusts were not associated with reduced rates of respiratory water loss. Our data suggest that longer cycle and interburst durations are a consequence of an evolved increased ability to store water, and thus buffer accumulated CO<sub>2</sub>, rather than an adaptive response to desiccation stress. Significantly lower cuticular water loss rates in the selected locusts confirm a more plausible adaptive response to desiccation conditions, considering the relatively low contribution of respiratory losses to the total evaporative water loss (~7% in the migratory locust). We conclude that DGCs are unlikely to be an evolutionary response to dehydration challenge in locusts.

## A5.17 CONVERGENT CAPACITIES OF NEUROHYPOPHYSIAL PEPTIDES ON ACID-BASE REGULATION IN CUTTLEFISH (*SEPIA PHARAONIS*)

WEDNESDAY 6 JULY, 2016 POSTER SESSION

YU-CHI CHEN (NATIONAL TAIWAN NORMAL UNIVERSITY, TAIWAN), PEI-HSUAN CHOU (NATIONAL TAIWAN NORMAL UNIVERSITY, TAIWAN), YUNG-CHE TSENG (NATIONAL TAIWAN NORMAL UNIVERSITY, TAIWAN)

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Cephalopods were proved to cope with hypercapnia predicament via efficient acid-base regulation in epithelium. In this study, we used embryos of cuttlefish, *Sepia pharaonis*, to examine integrative expressions of the neurohypophysial peptides, pro-sepiatocin and sepiatocin, and their putative receptor (sepiatocin-related receptor, spr) under CO<sub>2</sub>-induced acidic perturbation. RNA *in situ* hybridization images showed that spr were expressed in embryonic epithelium and adult gills, the dominant sites for acid-base

regulation. RNA signals of pro-sepiatocin and sepiatocin were both observed to be spatially co-expressed with ZN12 (a typical neuron marker) in optic lobe region. Transcripts of sepiatocin, pro-sepiatocin and spr were upregulated accompanied with those epithelial acid-base regulation candidates (e.g. *vha*, *nbc*, *nhe3*, *rhp* and *nka*) along CO<sub>2</sub>-exposed perturbations. Consequently, the present work inferred that the promptly activations of neurohypophysial-related peptides in molluscan cephalopod nervous system may convergent operate epidermal ion fluxes as vertebrates; accordingly, in order to cope with acid-base disturbances during their oviparous development, cephalopod embryos have evolved sophisticated evolution pathway regarding epithelium differentiation and the neurohypophysial hormones regulation.

## A5.18 THE IMPACT OF OCEAN ACIDIFICATION IN SEA BREAM INTESTINAL PHYSIOLOGY

WEDNESDAY 6 JULY, 2016 POSTER SESSION

SILVIA F. GREGORIO (CENTRE OF MARINE SCIENCES-CCMAR, PORTUGAL), I. RUIZ-JARABO (CENTRE OF MARINE SCIENCES-CCMAR, PORTUGAL), JUAN FUENTES (CENTRE OF MARINE SCIENCES-CCMAR, PORTUGAL)

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Previous studies demonstrated that ocean acidification threatens zooplankton recruitment suggesting that marine food webs are in danger. Marine fish are suggested to contribute to the carbon cycle as they cycle around 15% of the oceanic carbon, producing mineralized intestinal aggregates generated as by products of their osmoregulation. The formation of intestinal aggregates is necessarily driven by two factors: high divalent ion concentration and the required alkalization of the intestinal fluid to precipitate calcium carbonate.

Here we aimed at characterising the control of intestine aggregate production in the intestine of the gilthead sea bream (*Sparus aurata*), in response to increased relevant concentrations of environmental CO<sub>2</sub>. Our results confirmed that hypercapnia (800 and 1200 μatm CO<sub>2</sub>) elicits higher intestine epithelial HCO<sub>3</sub><sup>-</sup> secretion (BCS) and intestinal carbonate aggregates formation. Expression analysis revealed the up-regulation of crucial transport mechanisms involved not only in the intestinal BCS cascade (Slc4a4, Slc26a3 and Slc26a6) of sea bream, but also in other mechanisms involved in intestinal ion uptake and water absorption such as NKCC2 and the Aquaporin 1b. These results highlight the important role of fish in marine carbon cycle, as they contribute with intestinal biomineralization processes. And provides further evidences of the link of physiology, ion movements, water absorption and bicarbonate secretion in fish intestine.

Funded by FCT Portugal PTDC/MAR-BIO/3034/2014

### A5.19 CHANGES IN BRANCHIAL $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ CO-TRANSPORTER (NKCC) AND $\text{Na}^+/\text{K}^+$ -ATPASE $\alpha$ -SUBUNIT EXPRESSIONS OF PERSIAN STURGEON (*ACIPENSER PERSICUS*) JUVENILES DURING SHORT-TERM SALINITY TRANSFER

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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The short-term effect of abrupt salinity transfer from freshwater (FW) to the Caspian Sea water (CSW, 11‰) was investigated in 2-3 g ( $2.55 \pm 0.41$  g) Persian sturgeon, *Acipenser persicus*, juvenile. Immunolocalization of  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  (NKCC) Co-transporter and  $\text{Na}^+/\text{K}^+$ -ATPase (NKA), NKA activity and NKA  $\alpha$ -subunit mRNA expression has been studied in 0, 3, 6, 12, 24, 48 and 96 hours after abrupt transfer from FW to CSW. We reported for the first time, immunolocalization of NKCC which is co-localized with NKA throughout the chloride cells in the gill epithelium of Persian sturgeon after transfer to CSW. A partial sequences of the NKA  $\alpha$ -subunit (632bp) were described for this species. Its expression levels appeared almost unchanged throughout the experimental period compared to FW values. However, NKA activity was sharply increased in CSW by almost 2.8-fold ( $p < 0.05$ ) up to 96 hours after transfer. It has been demonstrated that Gill NKCC co-transporter abundance increased coinciding with increased gill NKA activity because of transfer from FW to CSW. The increased activity of NKCC during salt excretion in CSW may lead to an influx of  $\text{Na}^+$  into the chloride cells. Consequently, NKA activity increases for maintenance intracellular  $\text{Na}^+$  homeostasis.

### A5.20 WILL OSMOTIC CHALLENGE PREVENT DISPERSION OF THE INVASIVE CHICLID AUSTRALOHEROS FACETUS IN RIVERS OF SOUTHERN PORTUGAL?

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Ability to surpass osmoregulatory challenges posed by environmental salinity can determine the colonizing success of invasive species. The neotropical cichlid *Australoheros facetus* is invasive in southern Portugal, in small rivers subjected to flash floods that may drag fish downstream to estuaries. We performed experiments to determine the physiological mechanisms behind osmotic acclimation, growth and reproductive performance and social and parental behaviour in fish reared in a range of salinities. Set 1: fish (4-6cm,  $2 \times N = 30$ ) were reared for 90-days at 0-6-12-18ppt, weighted every 15-days and sampled every 30-days. Set 2: fish (9-

11cm,  $3 \times N = 60$ ) were subjected to an increase of 3ppt each 3-days. After 5-days at 0-6-12-18ppt, blood and tissues were collected and four social groups (5 fish/group at 0-6-12ppt) were formed and observed for one week for behavioural analyses. In set 1 growth reduction was obvious after 30-days at 18ppt ( $p < 0.05$ ). Aggressive behaviour was absent and mortality reached 56% in this group. In set 2 survival rate was only 25% after 5-days at 18ppt. Muscle water content, plasma lactate and protein decreased while osmolality, chloride and glucose increased at 18ppt in relation to control group. No salinity-related differences in plasma substrates or electrolytes (glucose, lactate and chloride), aggressive interactions, territorial status or dominance index were observed in other groups.  $\text{Na}^+/\text{K}^+$ -ATPase increase above the isosmotic point, and this demand may result in reduced growth and activity. Such conditions may lead to exhaustion, altered allocation of energy and disruption of behaviour, not allowing this species to survive for long periods in estuarine environments.

### A5.21 THE CHALLENGE WITHIN - RESPONSE TO HIGH AND LOW DIVALENT ION CONCENTRATION IN THE SEA BASS INTERNAL MILLIEU

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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The regulation of calcium balance in fish has mainly been associated to the hypocalcemic Stanniocalcin (STC), and more recently to the hypercalcemic Parathyroid Hormone related Protein. However, novel endocrine factors, such as Fibroblast growth factor FGF23, ion-sensing and transporting mechanisms have been associated to the balance of divalent ions in mammals and fish. To study the regulatory processes responsible for the maintenance of hydromineral homeostasis of divalent ions we exposed several sets of European sea bass, *Dicentrarchus labrax*, to altered environmental salinities or injected with saline solutions containing increased concentrations of calcium, magnesium and phosphate. Fish were sampled after 10 days at 0ppt, 12ppt and 35ppt, or before and 4 and 24 hours after injection. Plasma and urine were analysed for changes in osmolality, total and ultrafilterable electrolytes and endocrine profiles. Gill, kidney and intestinal samples were used for determination of enzymatic activity and gene expression and pituitary and corpuscle of Stannius for the expression and secretion of selected hormones and receptors. Salinity acclimation changed circulating cortisol and modulated branchial  $\text{Na}^+/\text{K}^+$ -ATPase activity, expression of branchial and renal ion and water transporting proteins and pituitary prolactin endocrine glands. Injection clearly evoked a transient increase of the administered ion, with changes in total and free fractions, protein levels and the relative ratios of Ca:Pi:Mg causing significant modifications in FGF23, CaSR and STC, and the expression of ion-transporting mechanisms. Further parameters are under evaluation and we hope to obtain a clearer picture of the several elements involved in ion homeostasis in fish.

## A5.22 THE SHARK CHOROID PLEXUS AND PHOSPHATE TRANSPORT: AN EXTREME MODEL TO STUDY THE HYDROMINERAL BALANCE AT THE BLOOD-CSF INTERFACE

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Inorganic phosphate (Pi) availability in water is scarce and the food is the only available source for fish. Despite its paramount importance, there is little information on overall phosphate endocrine sensing mechanisms controlling balance via intestinal and renal transporters. This study aimed to characterize the possible role of the choroid plexus (CP) in determining CSF [Pi]. The shark sheet-like IVth CP is was mounted in Ussing chambers. Under short-circuited conditions  $^{33}\text{P}$  fluxes revealed potent active CSF-to-blood transport with biochemical properties consistent with PiT Na<sup>+</sup>-dependent transporters (SLC20 family). RT-PCR revealed PiT1 and PiT2, but no NaPiII (SLC34 family) gene expression in CP. Immunohistochemistry localized PiT2 in the CP apical microvillar membranes while PiT1 occurred primarily in vascular endothelial cells. Active removal of Pi may adjust the CSF buffering capacity by maintaining a high sensitivity to small shifts in  $\text{CO}_2/\text{HCO}_3^-$ . Studies showed changes in [Pi]CSF may have dramatic effects on renal Pi excretion and Pi appetite in rats. Shark CP expresses both PTHrP and its receptor, endocrine players in fish Ca/Pi balance. Could choroidal epithelial PiT2 be part of a central Pi-sensing/regulatory pathway?

# A6 MOVING TO FEEDING: APPLYING MUSCLE-MECHANICS PRINCIPLES FROM LIMB TO FEEDING SYSTEMS

**ORGANISED BY: DR NICOLAI KONOW (HARVARD UNIVERSITY, UNITED STATES)  
& DR NICHOLAS GIDMARK (KNOX COLLEGE, UNITED STATES)**

## A6.1 COMPARING FEEDING AND LOCOMOTOR SYSTEM DESIGN. HOW DOES SELECTION WALK AND CHEW GUM AT THE SAME TIME?

📅 THURSDAY 7 JULY, 2016 ⌚ 09:05

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Locomotor and feeding systems deploy essentially the same tissues (muscle, bone, nerve, tendon, ligament) to perform very different functions. How are differences in design achieved within these 'constraints' and what do comparisons of locomotion and feeding systems tell us about principles of musculoskeletal design? Locomotor systems expend energy transporting the organism whereas feeding systems acquire energy, suggesting differences in the degree to which energetic efficiency is an optimality criterion. Both systems employ cyclic musculoskeletal movements; however, the dynamic properties of the muscles and neural control are arguably more important and pendulum mechanics less important in mammal chewing than mammal locomotion systems. In mammal feeding systems, optimization of displacement and force control is probably more important than energetic efficiency and speed, whereas the reverse is probably true in locomotion systems. Feeding and locomotion systems also differ in the relationships of kinetics to kinematics. During locomotion, the limbs and trunk generate substrate reaction forces in the 'middle' of the kinematic movement, e.g., with continued limb movement in the same direction prior to recovery. In contrast, biting and chewing reaction forces are generated at the end of closing, with a hard stop, followed by a recovery phase. We evaluate whether these differences are associated with variation in neural control systems, bony and muscle morphology, and connective tissue/tendon, and discuss the potential implications of these different system components on muscle dynamics during feeding.

## A6.2 FUNCTIONAL COMPARISON OF CHEWING AND BITING DEVICES IN ARTHROPODS

📅 THURSDAY 7 JULY, 2016 ⌚ 09:40

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Many arthropod species employ chewing mouth parts. Bite force measurements of the mandibles, i.e. the strongest mouth parts in these species, are crucial for the understanding of prey capture, feeding and many other behavioural traits. Due to technical limitations, however, bite force measurements over the whole angular range of the mandibles are extraordinary rare. Recent examinations in cockroaches in conjunction with previously published results now enable first comparative examinations of biting in a variety of differently adapted arthropod orders such as insects, decapod crustaceans and solifuges. Mandible closer muscles of insects are characterised by the occurrence of fast and slow muscle fibres. In omnivore cockroaches, carnivore ground beetles and in many ant species slow muscle fibres contribute significantly to overall bite forces, in particular when chewing on tough and resilient food. However, in stag beetles, who use their oversized mandibles primarily in conspecific fights for mating opportunities, bite forces seem to be dominated by fast muscle fibres. In contrast to the pincers of crustaceans and scissor like chelicerae of solpugids, mandibles are independently driven by external muscles and work against each other. Though not increasing maximum attainable bite forces such an arrangement reduces necessary muscle shortening and contraction speed. Therefore, the closer muscles can work closer to their optimum fibre length and contraction speed. Consequently increased bite efficiency most likely facilitates sustained chewing on tough food items.

## A6.3 FROM LOCOMOTION TO FEEDING: WHERE DOES FIBER TYPE FIT IN?

📅 THURSDAY 7 JULY, 2016 ⌚ 09:55

👤 ANDREA B TAYLOR (DUKE UNIVERSITY, UNITED STATES),  
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Fiber phenotype is an important determinant of the contractile properties of a muscle fiber. In vertebrates, fiber type composition of the locomotor muscles has been shown to fine tune muscles for specific motor tasks, such as steady-state swimming and distance running, as well as faster more powerful tasks such as burst

swimming and jumping. Fiber types and their myosin heavy chain (MHC) composition have been extensively studied in mammalian locomotor muscles. They typically comprise four fiber types that have been classified into two basic phenotypes, slow/fatigue resistant and fast/rapid fatigue. Jaw-adductor fiber types have been investigated in non-primate mammals and by contrast, display a much greater diversity of MHC content, higher prevalence of hybrid fibers, and perhaps a greater degree of clade-specific plasticity. This variation in MHC content suggests a high degree of functional diversity in ways that may serve to fine tune feeding performance. Primates vary widely in feeding behavior and thus provide an excellent framework for understanding this functional diversity. Here we review some of the fiber type work on vertebrate locomotor and feeding musculature and present novel data on fiber phenotype of the jaw adductors in adult sooty mangabeys (*Cercocebus atys*). We show that *C. atys* have a predominantly fatigue resistant jaw-adductor phenotype compared to macaques and baboons. We discuss these findings in the context of their habitual feeding on resistant objects and address how integration of fiber phenotype could improve understanding of feeding-system muscle mechanics.

## A6.4 USING MULTIBODY DYNAMIC ANALYSIS FOR STUDYING MASTICATION IN THE MOUSE

THURSDAY 7 JULY, 2016 10:10

ANNA CHABOKDAST (UNIVERSITY OF OXFORD, UNITED KINGDOM), SAMUEL COBB (THE HULL YORK MEDICAL SCHOOL, UNITED KINGDOM), MICHAEL J FAGAN (UNIVERSITY OF HULL, UNITED KINGDOM), ANTHONY HERREL (MUSÉUM NATIONAL D'HISTOIRE NATURELLE, FRANCE)

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Multibody dynamic analysis (MDA) techniques, which were originally developed for engineering applications, have recently been used to study biological systems such as the masticatory system of extinct and extant species. MDA is an ideal tool for the investigation of the three-dimensional dynamic biomechanics of such a complex biological system with multiple layers and orientations of muscles. The mouse dentition only has incisors and molars, which are separated by a large gap, and have distinct functions. The incisors use a simple movement in a single plane to gnaw, whereas the molars use more complex movements including lateral excursions to chew. We used MDA techniques to study the maximum biting force of the adult mouse in these two biting scenarios. In addition, since muscle recruitment is important in functional and developmental studies, we were also interested in the recruitment pattern that the mouse uses in each of those masticatory functions. For these purposes, we developed individual-specific models that included accurate muscle volume data and orientations obtained from micro-CT scans of the specimens. To validate our model, we compared the estimated bite force from the models with experimental measurements of voluntarily bite force at the incisors of the same specimens prior to sacrifice and found comparable values of the bite forces (9.01 Vs 8.97 N respectively). Moreover, we estimated that, for an equal bite force and gape, the muscle orientation patterns are different in the two biting activities, with the highest difference occurring in the masseter, the largest masticatory muscle.

## A6.5 USING 'MUSCULO-ROBOTIC' METHODS FROM FROG LOCOMOTION TO UNDERSTAND FISH SUCTION FEEDING

THURSDAY 7 JULY, 2016 10:55

CHRISTOPHER T RICHARDS (THE ROYAL VETERINARY COLLEGE, UNITED KINGDOM), ARIEL CAMP (BROWN UNIVERSITY, UNITED STATES)

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Frog legs and fish mouths share some things in common: Each perform extremely rapid behaviors crucial for survival. Each demand enormous mechanical power approaching - or sometimes exceeding - the limits of muscles. Accordingly, each system has served as a model determining how muscle physiology limits performance. However, muscle properties alone do not guarantee effective performance. Rather, it is the coupling of anatomical, muscular and environmental (e.g. fluid dynamic) properties which interact to govern biomechanical behavior. Hence, in either taxon a broad aim is to learn how muscle dynamics respond to evolutionary changes in anatomy (and vice versa). Given these parallels, we propose how recently developed techniques for swimming frogs may apply to fish suction feeding. Our goals are: 1) To use a simple computational model to explore the influence of musculoskeletal dynamics and mouth geometry on suction feeding performance. 2) To propose a design for a bio-robotic fish mouth model 'remote controlled' by in vitro muscle tissue to test computational predictions. We hypothesize that changes in fish mouth shape (with muscle parameters unchanged) will affect the fluid interactions and therefore strongly influence muscle power output and suction feeding performance. Likewise, we expect that altering muscle kinetic properties will influence which anatomical properties are 'optimal' for performance. Ultimately, these models will reveal general principles of acceleration-based non-cyclic motions that can be applied back to problems of locomotor biomechanics.

## A6.6 A POWERFUL PERSPECTIVE ON SUCTION FEEDING SHOWS THE IMPORTANCE OF BODY MUSCLES FOR FEEDING IN FISH

THURSDAY 7 JULY, 2016 11:25

ARIEL L CAMP (BROWN UNIVERSITY, UNITED STATES), ELIZABETH L BRAINERD (BROWN UNIVERSITY, UNITED STATES), THOMAS J ROBERTS (BROWN UNIVERSITY, UNITED STATES)

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Vertebrate feeding and locomotion behaviors depend on the musculoskeletal system to produce fast and forceful movements. Feeding studies often use force measurements alone to link muscle function to feeding performance, but muscle force can be altered by skeletal gearing and therefore be difficult to directly relate to feeding force. An alternative approach used in many locomotion studies is to measure the mechanical energy (work) and rate of energy production (power). A muscle's maximum work and power are ultimately limited by its mass, and are unaltered by skeletal gearing. However, power can be amplified by elastic structures that slowly store muscle energy

and then release it rapidly. Therefore, work and power measurements have been used to examine the relative contributions of muscles and elastic elements to generating the power required for locomotion. To understand if similar mechanisms are used for powerful feeding, more studies are needed of work and power during feeding. We recorded work and power in a suction feeding fish to re-examine the role of the head and body muscles, using X-ray imaging (XROMM and fluoromicrometry). Head muscles produced little of the power or work of suction feeding, due to their small size and limited shortening. Instead of amplifying the head muscles' power with elastic elements, like other high-power vertebrate feeders, these fish relied on the large body muscles to power suction. These results emphasize the importance of the body muscles for feeding and swimming in fish, and contribute to our understanding of power production across feeding and locomotion.

### A6.7 THE NEED FOR SPEED: FUNCTIONAL SPECIALIZATIONS OF LOCOMOTOR AND FEEDING MUSCLES IN *ANOLIS* LIZARDS

THURSDAY 7 JULY, 2016 13:50

CHRISTOPHER V ANDERSON (BROWN UNIVERSITY,  
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Feeding and locomotor performance involve different mechanical demands. Variation in muscle contractile properties between locomotor and feeding muscles may be shaped by their differing functions, however few such comparisons have been made. We hypothesized that due to the rapid force generating events associated with sprinting, limb muscles would be faster than jaw muscles. To test this prediction, we examined the contractile physiology of a locomotor and a feeding muscle among five species of *Anolis* lizards. Consistent with our hypothesis, twitch times were faster in locomotor muscles than feeding muscles for all species. However, peak contractile velocity ( $V_{max}$ ) was significantly faster in jaw muscles than locomotor muscles. Further, jaw muscle force-velocity relationships exhibited greater curvature and lower power ratios than leg muscles, resulting in power being maximized at lower normalized contractile velocity in jaw muscles. While variation in the speed of muscle based on differing fiber types may be expected, our results indicate that different measures of speed (i.e., activation vs. shortening velocity) do not always covary. In *Anolis*, limb muscles can turn on and off more quickly than jaw muscles, but jaw muscles shorten at higher relative speeds. Thus, muscle properties may be shaped not just by association with fast or slow movements, but also by the kind of speed needed. Whether these differences result from differences in the mechanical demands of running vs. biting, or from constraints on the morphology of muscles and bony lever systems, remains to be determined. This research was supported by NSF grant IOS 1354289.

### A6.8 THE FORCE-LENGTH RELATIONSHIP OF SKELETAL MUSCLE AS A BIOMECHANICAL ADAPTATION TO TROPHIC NICHE IN SALMONID FISHES

THURSDAY 7 JULY, 2016 14:30

NICHOLAS J GIDMARK (KNOX COLLEGE, UNITED STATES),  
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Functional specializations for a particular feeding niche could be reflected in skeletal anatomy, muscular anatomy/physiology, or both. Skeletal specializations have been a major focus of functional morphology literature, especially in fish feeding systems. Here, we explore variation in muscle morphology in two species of salmonid fishes that are sympatric but differ in prey specialization. King salmon (*Oncorhynchus tshawytscha*) eat small, fast fish, and pink salmon (*Oncorhynchus gorbuscha*) primarily filter feed on planktonic organisms, keeping their mouths open while swimming. Salmon close their jaws using the adductor mandibulae, which, like all skeletal muscles, is constrained by a strict relationship between muscle length and force. Muscles that are over-stretched or over-shortened exert weaker forces than they do at optimal length, and muscle length corresponds to gape. We compared king and pink salmon by measuring the force-length curves of their adductor mandibulae and demonstrated that in king salmon, maximum bite force is achieved close to maximum gape (67% of max gape,  $n=3$ ). This may allow them to take advantage of optimal muscle length, and thus greater force production, when eating large or elusive prey. In pink salmon, the force-length curve is centered at a smaller relative gape that is closer to mid-gape (43% of max gape,  $n=6$ ). This may facilitate filter feeding, allowing reasonably high forces at all gapes. Optimal gapes were significantly different between species ( $p=0.0282$ ), indicating that feeding preferences correlate with differences in jaw muscle physiology, resulting in distinct optimal solutions to the force-length constraint.

## A6.9 CAN MUSCLE REDUNDANCY IN THE JAW SYSTEM OF LIZARDS BE EXPLAINED BY THE FUNCTIONAL ROLES OF MUSCLES ACTING AS MOTORS OR BRAKES?

THURSDAY 7 JULY, 2016 14:45

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It is well known that muscles can either act as motors or as brakes in complex multi-segment systems during locomotion. The most common occurrence of this pattern is an agonist-antagonist co-contraction allowing one muscle to absorb energy during active lengthening while the other muscle undergoes active shortening. In some cases such as during running in cockroaches this is also observed among agonistic muscles. The differential roles of muscles acting alternatively as motors or brakes helps them stabilize joints and resist perturbations during running. These complex patterns of activation may explain the large degree of redundancy commonly observed in musculoskeletal systems. The jaw system in lizards is one of the most highly redundant musculo-skeletal systems with over ten muscle bundles acting across a single joint. Here, I explore whether this redundancy could be associated with divergent functional roles of the different muscle bundles acting as motors or brakes respectively as is commonly observed in locomotor muscles. I analyzed data on muscle activation patterns for six species of lizards and quantify the occurrence of co-contractions between depressor and levator muscles. In doing so I predict that this should be more common in lizards with mobile jaw joints if this phenomenon plays an important role in jaw stabilization. Moreover, I explore whether active lengthening could take place in the jaw adductors. The results show that agonist-antagonist co-contractions are common and that active lengthening may indeed take place. These results may provide some explanation for the complexity of the jaw system in lizards.

## A6.10 LINKING MUSCLE MECHANICAL VERSATILITY WITH PATTERNS OF SHAPE-CHANGE AND CONTRACTILE PROPERTIES IN VERTEBRATE FEEDING SYSTEMS

THURSDAY 7 JULY, 2016 15:00

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Studies of limb muscle function have highlighted how interactions between muscle shape-change and contractile properties allow a given muscle to act alternatively as a motor, strut or brake during locomotion. Jaw and hyoid muscles have diverse architectures but it remains unclear how muscle architectural and contractile dynamics interplay to shape feeding performance. Vertebrate jaw mechanics vary substantially between crushing bites, super-fast strikes, and slow food-processing excursions, indicating that jaw muscle contractions are tuned to demands for force, speed, power, and work production. Elastic recoil and contractile shape-change has been shown to extend the operating ranges of pennate limb muscles but similar phenomena have only been identified in a few jaw muscles. Data on vertebrate hyoid systems suggest that muscle passive properties are important for shaping system posture and function, analogous to recent findings for anuran hopping. Hyoid muscles undergo surprisingly complex contractions given their parallel-fibered architecture. Evidence of regional heterogeneity in activation and length-change within a muscle may reflect hyoid motion-dampening, whereas changes from muscle shortening to lengthening across behaviors facilitate explosive power-transmission in one cycle and small, precisely repeated movements in subsequent cycles. The common occurrence of sternohyoid lengthening contractions over broad ranges of its length-tension curve may aid power-transfer from body muscles as force-enhancement arises from lengthening force-velocity effects. Jaw and hyoid muscle activation and contractions are tightly sequenced, or near-simultaneous in many feeding behaviors, which highlights the importance of studying their integrated function in order to significantly further our understanding of feeding system dynamics.

## A6.11 WHAT MIGHT STUDIES OF LIMB MUSCLE MECHANICS TEACH US ABOUT FEEDING SYSTEMS?

📅 THURSDAY 7 JULY, 2016 ⌚ 15:45

👤 THOMAS J ROBERTS (BROWN UNIVERSITY, UNITED STATES)

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Our understanding of the link between muscle function and locomotor performance rests on a foundation of simple models. Walking is modeled as an inverted pendulum, and running as a spring-mass system, and these simple mechanical analogs organize our understanding of the metabolic energetics of movement, the role of elastic mechanisms, and the significance of muscle contractile characteristics. Can we discover similar models for feeding mechanics, and if so, will they do us any good? To address this question it is constructive to review 1) what motivated the development of simple models for terrestrial locomotion, 2) how these models advance our understanding, and 3) whether the utility of such models is somehow unique to studies of locomotion. In many cases models of locomotor mechanics were motivated by a desire to explain metabolic energy cost, which exhibits simple empirical patterns (e.g., the linear increase in cost with running speed), despite the complexity of muscle and limb function. The models identify high-level mechanical tasks, such as maintaining body support or cycling mechanical energy, that lead to predictions about how individual muscles function. These predictions have helped us to understand that muscles perform several mechanical tasks during movement, acting as struts, brakes, motors and springs. They have also helped us determine the implications of characteristic muscle contractile behavior, such as length-tension and force-velocity relationships, for muscle function in locomotion. It seems likely that similar models would benefit our understanding of feeding mechanics.

# A7 SHORT RANGE VISUAL GUIDANCE IN BIRDS

**ORGANISED BY:** PROF DOUGLAS ALTSHULER (UNIVERSITY OF BRITISH COLUMBIA, CANADA) AND PROF MANDYAM V SRINIVASAN (UNIVERSITY OF QUEENSLAND, AUSTRALIA)

**SESSION SPONSORED BY:** THE COMPANY OF BIOLOGISTS

## A7.1 VISUAL GUIDANCE OF FLIGHT: BEES VERSUS BIRDS, AND APPLICATIONS TO UAVS

📅 MONDAY 4 JULY, 2016 ⌚ 11:00

👤 MANDYAM V SRINIVASAN (UNIVERSITY OF QUEENSLAND, AUSTRALIA), INGO SCHIFFNER (UNIVERSITY OF QUEENSLAND, AUSTRALIA), HONG D VO (UNIVERSITY OF QUEENSLAND, AUSTRALIA)

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Flying insects and birds are remarkably adept at seeing and perceiving the world and navigating effectively in it, despite possessing nervous systems that are much smaller than our own. This presentation will review our investigations of visually guided flight in honeybees – exploring, for example, how they use vision to control flight speed, negotiate narrow passages, and orchestrate smooth landings – and compare them with our findings with regard to similar questions in budgerigars. The aim is to uncover general principles of visual guidance that may be common to all airborne creatures, as well as to discover interesting species-specific differences. The presentation will conclude with a brief description of the ways in which we are translating some of these biological principles into the design of vision-based systems for the guidance of unmanned aerial vehicles.

## A7.2 WIND DRIFT COMPENSATION IN HOMING PIGEONS WHEN IN SIGHT OF THE LOFT

📅 MONDAY 4 JULY, 2016 ⌚ 11:40

👤 JAMES A WALKER (UNIVERSITY OF OXFORD, UNITED KINGDOM), GRAHAM K TAYLOR (UNIVERSITY OF OXFORD, UNITED KINGDOM)

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When flying in a moving air mass, birds must make adjustments to their heading vector to minimise lateral displacement and arrive at their desired goal. The compensation for wind drift has been widely studied in migrating birds but has received relatively little attention for birds guiding their trajectory over shorter journeys. To address this, GPS-derived tracks were analysed from homing pigeons released within sight of their destination along with detailed wind data to explore the visual mechanisms used by the

birds to compensate for the effect of wind. We found that instead of applying a continuous wind drift correction, pigeons follow curved trajectories which are displaced laterally in the direction of the cross wind experienced. A simple delayed compensation model is proposed for drift correction which requires a threshold lateral displacement to be reached before a heading adjustment is made. This threshold is detected using the relative angular movement of distant landmarks across the pigeon's visual field.

## A7.3 AVOIDING OBSTACLES DURING FLIGHT: BIRD NAVIGATION THROUGH CLUTTERED ENVIRONMENTS

📅 MONDAY 4 JULY, 2016 ⌚ 11:55

👤 ANDREW A BIEWENER (HARVARD UNIVERSITY, UNITED STATES), IVO ROS (CAL. TECH., UNITED STATES), HUAI-TI LIN (HHMI JANELIA FARMS, UNITED STATES), C. DAVID WILLIAMS (HARVARD UNIV., UNITED STATES), PARTHA BHAGAVATULA (HARVARD UNIV., UNITED STATES)

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The ability to fly through cluttered environments is essential to the ecological and evolutionary success of many groups of flying animals. Flight navigation and obstacle avoidance require exceptional aerodynamic maneuvering performance coordinated by rapid processing of visual cues. We previously examined strategies that pigeons adopt to fly past vertical obstacles, in which flight guidance was well described by steering control that targeted gap openings between nearby obstacles. We observed that pigeons bias their flight direction toward larger visual gaps when making fast steering decisions. We also found that pigeons adopt discrete wing morphing strategies to traverse vertical obstacles of varying gap width. In recent experiments, 3D flight kinematics was recorded as pigeons flew through randomized distributions of a 20 horizontal obstacle array. To negotiate horizontal obstacles, pigeons traded-off a decrease in kinetic energy to gain potential energy and decelerated to maintain greater control authority for negotiating the horizontal obstacles. Pigeons also decreased wing stroke amplitude and span to avoid wing contact with obstacles. As for flight past vertical obstacles, navigation through horizontal obstacles was similarly well described when an upward steering bias was added to a steering model that selects larger visual gaps between approaching nearby obstacles. These results show that pigeons exhibit a remarkable kinesthetic sense of body and wing position that they use to maintain their control authority when flying past dense arrays of obstacles.

## A7.4 WHAT DRIVES AVIAN VISION?

📅 MONDAY 4 JULY, 2016 ⌚ 12:25

👤 GRAHAM MARTIN (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM)

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A comparative analysis of information about the vision of birds, particularly retinal and visual field topography, and its key functions. The main conclusion is that in birds vision is driven primarily by the information required for the accurate and precise control of the position and time of arrival of the bill at a target. These requirements are traded-off against competing informational demands for the detection of predators. A third driver is the need to avoid imaging the sun upon the retina in species with large eyes. It is shown that vision in birds is finely tuned to these informational demands and that differences exist between closely related species which reflect subtle differences in the perceptual challenges that the birds face in their foraging. It is proposed that the control of locomotion is achieved within constraints upon vision set by these key drivers. It is concluded that from a sensory ecology perspective a bird is best characterized as "a bill guided by an eye".

## A7.5 HOVERING HUMMINGBIRDS ATTEMPT TO STABILISE VISUAL MOTION DURING DOCKED FEEDING

📅 MONDAY 4 JULY, 2016 ⌚ 13:55

👤 BENJAMIN GOLLER (UNIVERSITY OF BRITISH COLUMBIA, CANADA), DOUGLAS L ALTSHULER (UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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Hovering is a behaviour that requires the flying animal to maintain a stable position in mid-air, a balancing act that presents both a motor and a sensory challenge. Hummingbirds are the only birds specialised for sustained hovering, which allows them to feed on the nectar of delicate flowers. We know much about how hummingbird physiology, anatomy, and wing movements enable hovering, but how do hummingbirds use their senses to feed on the wing? We have previously shown that vision is critical to a flying hummingbird's ability to maintain positional stability in the laboratory and that they respond to moving patterns even when large portions of the visual field are devoted to stationary patterns. Natural landscapes frequently have potentially disruptive moving features, so we hypothesised that feeding hummingbirds would use bill contact with a food source to override their hovering response to moving visual patterns. We developed an instrumented feeder that could measure forces applied by a feeding hummingbird to the nectar reservoir. The feeding birds were then shown visual stimuli and we measured their responses during feeding. Surprisingly, hummingbirds feeding on the wing still responded to visual patterns by pushing against the feeder, primarily along the horizontal axis. The response quickly saturated at slow pattern motion speeds. These results show that hummingbirds attempt to stabilise visual motions even when docked at a rigid nectar source. How tactile and visual control facilitates hummingbird hover feeding in a natural environment with flowers on flexible stems has yet to be investigated.

## A7.6 VISUAL GUIDANCE OF FORWARD FLIGHT IN HUMMINGBIRDS REVEALS CONTROL BASED ON IMAGE FEATURES INSTEAD OF PATTERN VELOCITY

📅 MONDAY 4 JULY, 2016 ⌚ 14:15

👤 DOUGLAS L ALTSHULER (UNIVERSITY OF BRITISH COLUMBIA, CANADA), ROSLYN DAKIN (UNIVERSITY OF BRITISH COLUMBIA, CANADA), TYEE K FELLOWS (UNIVERSITY OF BRITISH COLUMBIA, CANADA)

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Information about self-motion and obstacles in the environment is encoded by optic flow, the movement of images on the eye. Decades of research have revealed that flying insects control speed, altitude, and trajectory by a simple strategy of maintaining or balancing the translational velocity of images on the eyes, known as pattern velocity. It has been proposed that birds may use a similar algorithm but this hypothesis has not been tested directly. We examined the influence of pattern velocity on avian flight by manipulating the motion of patterns on the walls of a tunnel traversed by Anna's hummingbirds. Contrary to prediction, we found that lateral course control is not based on regulating nasal-to-temporal pattern velocity. Instead, birds closely monitored feature height in the vertical axis, and steered away from taller features even in the absence of nasal-to-temporal pattern velocity cues. We also observed that birds adjusted their flight altitude in response to upward motion of the horizontal plane, which simulates vertical descent. Collectively, our results suggest that birds avoid collisions to the sides and below by monitoring expansion and translational velocity in the vertical axis. This distinct algorithm may derive from greater need to avoid collisions in birds, as compared to small insects.

## A7.7 OPTIMISATION OF EYE HEIGHT FOR LOCOMOTION CONTROL

📅 MONDAY 4 JULY, 2016 ⌚ 14:35

👤 J BURN (UNIVERSITY OF BRISTOL, UNITED KINGDOM),  
KATHERINE A J DANIELS (UNIVERSITY OF BRISTOL,  
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Vision is an important sensory pathway for information used to control locomotion.

A number of studies using different approaches suggest that, during walking, terrestrial animals acquire detailed information about the ground when it is at a distance ahead of approximately two steps. Using projective geometry we showed that a vision system that is optimally configured to maximise the resolution of information about the ground at a given distance ahead requires the eyes to be at a certain unique height above the ground. Above and below the optimum height resolution is lost due to increased distance to the ground and an increasingly acute projection of the ground onto the retina respectively. We hypothesised that eye height would be associated with step length in terrestrial animals during walking. Data for 20 species were obtained from plates published by Edward Muybridge in 1899 and stills taken from wildlife documentaries. The eye height of humans closely approximated the optimum predicted by the model. In other species we found no evidence of a systematic relationship. With the exception of humans, the species used for the study were able to change eye height by altering neck angle. It is possible that these animals adjust eye height adaptively in response to changing demand for information about local ground conditions. The hypothesis that animals optimise eye height dynamically in response to demand for visual information is experimentally tractable and will be explored in future investigation.

## A7.8 NEURAL PATHWAYS SUBSERVING VISUAL CONTROL OF FLIGHT IN BIRDS

📅 MONDAY 4 JULY, 2016 ⌚ 14:50

👤 DOUG R WYLIE (UNIVERSITY OF ALBERTA, CANADA),  
CRISTIAN GUTIÉRREZ-IBÁÑEZ (TECHNISCHE UNIVERSITÄT  
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As a bird flies through an environment cluttered with numerous objects, both global and local visual motion will occur across the retina. The global motion will occur simply because any form of self-motion will induce optic flow across the retina. The local motion would be the motion parallax that occurs due to the fact that objects and surfaces are at different depths from the observer. In this paper we will review the properties of neurons in various pathways in the avian brain that make them candidates for the visual control of motion through a cluttered environment. We will make a case for a ponto-cerebellar pathway in this regard. The lateral pontine nucleus and posterior cerebellum (folia VI-VIII) integrate information from the nucleus lentiformis mesencephali, which analyzes optic flow, and the optic tectum, which analyzes local visual motion. Using a modern comparative approach, we will examine the relative size of the lateral pontine across a wide variety of birds. We expect that the relative size of the lateral pontine will be larger in species that live in environments that require obstacle avoidance during flight.

## A7.9 HOW BODY SIZE AFFECTS HEAD STABILIZATION IN FLYING BIRDS

📅 MONDAY 4 JULY, 2016 ⌚ 15:20

👤 DANIEL QUINN (STANFORD UNIVERSITY, UNITED STATES)

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Birds rely on vision to guide their flight, so they stabilize their head as they fly. The mechanisms that account for this head stabilization are poorly understood. We present data that show how lovebirds (*Agapornis roseicollis*) stabilize their head when flying in still air and in gusts with magnitudes comparable to their flight speed. Using a neck suspension model, we estimate the damping properties necessary to stabilize the head. We then verify the model with data obtained for birds spanning six orders of magnitude in body mass. Our analysis demonstrates how head kinematics scale with bird size and explains why head stabilization is a very different challenge for small birds like hummingbirds than it is for large birds like swans.

## A7.10 VISUALLY-GUIDED PURSUIT BEHAVIOUR IN THE HARRIS'S HAWK *PARABUTEO UNICINCTUS*

📅 MONDAY 4 JULY, 2016 ⌚ 16:10

👤 GRAHAM K TAYLOR (UNIVERSITY OF OXFORD, UNITED KINGDOM), CAROLINE H BRIGHTON (UNIVERSITY OF OXFORD, UNITED KINGDOM)

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Here we identify the guidance law used by Harris's Hawks *Parabuteo unicinctus* pursuing artificial targets. Five captive Harris's Hawks were filmed using four high-speed cameras, when flying at a lure towed along a zigzag course over the ground. We used multi-station photogrammetry to reconstruct the instantaneous positions of the bird and lure, and used a correlation technique to show that the birds flew directly at their targets, with a lag of approximately 0.2s between changes in the line of sight vector and turning of the bird's velocity vector. We tested the hypothesis that the birds used a guidance law known as proportional navigation, in which the pursuer turns at a rate proportional to the angular rate of the line of sight to its target (this is the guidance law used by peregrine falcons *Falco peregrinus* and most guided missiles). We used a prediction error method to identify the constant of proportionality, called the navigation constant, and found that the trajectories could be simulated by assuming unity navigation constant with a delay of 0.2s. This fitted value of the navigation constant is significantly lower than in falcons and missiles, which we hypothesise is an adaptation to pursuit in the cluttered environments favoured by hawks. Any attempt to fly an intercept trajectory in a cluttered environment is likely to lead to conflict between target-oriented and obstacle-avoidance behaviours, which will be avoided by following the track of the target through the clutter, as occurs in a pursuit with a navigation constant of one.

## A7.11 THE INFORMATION FOR GUIDING FLIGHT

📅 MONDAY 4 JULY, 2016 ⌚ 16:40

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A theory of guidance of flight will be presented, developed from General Tau Theory. The central idea is that guiding flight (or indeed any movement) to or from something is based on (a) perceptual information about  $\rho$  ( $=1/\tau$ ), the relative-rate-of-change of the physical gap between the animal and that something, and (b) intrinsically generated prescriptive  $\rho$ s, specifying how the  $\rho$  of the physical gap is intended to change.  $\rho$  information is, in principle, directly available in all perceptual modalities - vision, hearing, touch, olfaction, echolocation, electro-location, thermal-location. In contrast, distance and velocity information are not directly available. The goal of an action may be reach something, such as a perch, the ground, an opening in foliage, a mate or a prey; or it may be to avoid hitting obstacles or other animals, as when moving in a flock or swarm. Theory and experimental evidence on how such manoeuvres are accomplished will be described.

## A7.12 HEAD-BOBBING IN PIGEONS: VISION OR BIOMECHANICS?

📅 MONDAY 4 JULY, 2016 ⌚ 17:05

👤 NIKOLAUS F TROJE (QUEEN'S UNIVERSITY, CANADA), LESLIE M THEUNISSEN (QUEEN'S UNIVERSITY, CANADA)

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Head-bobbing during terrestrial locomotion is observed in many bird species. However, the functional significance of this behaviour is not clear at all. Current theories focus on visual functions: A visual input that is free of self-induced optic flow during the hold phase, and increased flow velocities that provide increased signal-noise ratios for motion-parallax measures during the thrust phase of the head. I will critically review the evidence for these theories and, using pigeons, I will present the results of experiments that failed to replicate earlier findings in their support. As an alternative, I will discuss two new theories and experimental support for them: The first concerns the possibility to monocularly estimate distance to objects and agents in situations in which normal motion parallax would not be able to provide information. The second is based on measurements of ground reaction forces during locomotion and suggests that head-bobbing reduces the metabolic costs associated with walking.

# A8 GENERAL BIOMECHANICS

**ORGANISED BY:** PROF PETER AERTS (UNIVERSITY OF ANTWERP, BELGIUM)  
AND PROF ROB JAMES (COVENTRY UNIVERSITY, UNITED KINGDOM)

## A8.1 LOCALISED FREQUENCY- AND AMPLITUDE-DEPENDENT MOTION IN THE LOCUST TYMPANUM: AN INVESTIGATION INTO ACTIVE PROCESSES IN AN INSECT EAR

📅 TUESDAY 5 JULY, 2016 ⌚ 10:30

👤 ELIZABETH KLENSCHI (UNIVERSITY OF STRATHCLYDE, UNITED KINGDOM), ROGER DOMINGO-ROCA (CENTRE FOR ULTRASONIC ENGINEERING UNIVERSITY OF STRATHCLYDE, UNITED KINGDOM), JEREMY S. GIBSON (CENTRE FOR ULTRASONIC ENGINEERING UNIVERSITY OF STRATHCLYDE, UNITED KINGDOM), JOSEPH C. JACKSON (CENTRE FOR ULTRASONIC ENGINEERING UNIVERSITY OF STRATHCLYDE, UNITED KINGDOM), JAMES F.C. WINDMILL (CENTRE FOR ULTRASONIC ENGINEERING UNIVERSITY OF STRATHCLYDE, UNITED KINGDOM)

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Insect tympanal ears are often considered to be among the simplest in the animal kingdom, and yet they have been shown to be capable of fine frequency analysis and signal amplification. In Caeliferans, the heterogeneous structure of the tympanal membrane has been shown to be crucial in ensuring efficient frequency analysis by inducing localised, frequency-dependent membrane displacement. In addition, studies have demonstrated that this system is capable of generating distortion-product otoacoustic emissions (DPOAEs), which are known to be characteristic of non-linear mechanical systems and are linked to active acoustic amplification in vertebrates. This study characterised and investigated the presence of DPOAEs in the ear of the desert locust *Schistocerca gregaria* and provided hypotheses explaining the source of these emissions and their function. Nanoscale 3-D measurements of membrane displacement confirmed that DPOAEs are present in this species, and that their localisation is characteristic of the known tonotopy of this system. Moreover, it was observed that the direction of membrane displacement is amplitude-dependent, with low amplitudes leading to this displacement occurring almost exclusively out of plane and higher amplitudes causing a partial shift to in-plane motion in areas where DPOAEs were observed. We suggest that this phenomenon is related to the generation of DPOAEs, and is a product of the intrinsic mechanical properties of the tympanum driven by the sensory receptors in this system. These results shed more light on the mechanical components responsible for signal detection and amplification in insect tympanal ears.

## A8.2 THE EFFECTS OF CRANIOKINESIS ON THE MIDDLE EAR OF DOMESTIC CHICKENS (*GALLUS GALLUS DOMESTICUS*)

📅 TUESDAY 5 JULY, 2016 ⌚ 10:45

👤 RAF CLAES (UNIVERSITY OF ANTWERP, BELGIUM), PIETER MUYSHONDT (UNIVERSITY OF ANTWERP, BELGIUM), LUC VAN HOOREBEKE (UNIVERSITY OF GHENT, BELGIUM), JELLE DHAENE (UNIVERSITY OF GHENT, BELGIUM), JORIS DIRCKX (UNIVERSITY OF ANTWERP, BELGIUM), PETER AERTS (UNIVERSITY OF ANTWERP, BELGIUM)

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The mammalian middle ear consists of an eardrum, three ossicles, two muscles and some ligaments, and is enclosed in a single bony structure. Ossicle movements, mediated by these muscles and ligaments, adapt sound transmission. The avian middle ear is seemingly simpler: an eardrum connected to one ossicle (columella), one muscle and some ligaments. This simplicity seems to constrain adaptation capabilities. We hypothesize, however, that craniokinesis may play a role in the adaptation of sound transmission as the avian middle ear is not enclosed by one rigid structure, but also by the quadrate and by soft tissue. The eardrum is connected to the movable quadrate. Craniokinetic movement of the quadrate may thus effect the eardrum as well as the columella. To test this, hens and roosters (*Gallus gallus domesticus*) are used as models that differ in vocalization capacity.  $\mu$ CT-scans were made of the heads of 3 hens and 3 roosters, with beaks closed and fully opened. A surface model was created to quantify quadrate motion, columella displacement and changes in the eardrum. Upper bill elevation was found to be greater in roosters which results from a greater frontal rotation of the quadrate. The quadrate movements do not result in significant displacements of the columella, but do effect the eardrum. The strain of the membrane changes with beak opening in both sexes, and there is a clear displacement of the membrane in roosters. Based on these results we assume that craniokinesis may play a role in the sound transmission of the bird.

### A8.3 SING ME AN OLD FASHIONED SONG: WING RESONANCES IN THE RELICT BUSH-CRICKET *CYPHODERRIS MONSTROSA* (INSECTA: ORTHOPTERA: PROPHALANGOPSIDAE)

TUESDAY 5 JULY, 2016 11:00

BENEDICT D CHIVERS (UNIVERSITY OF LINCOLN, UNITED KINGDOM), THORIN JONSSON (UNIVERSITY OF LINCOLN, UNITED KINGDOM), OLIVIER BÉTHOUX (MUSÉUM NATIONAL D'HISTOIRE NATURELLE, FRANCE), ANDREW C MASON (UNIVERSITY OF TORONTO, CANADA), FERNANDO MONTEALEGRE-Z (UNIVERSITY OF LINCOLN, UNITED KINGDOM)

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Male bush-crickets, grigs, and crickets produce acoustic signals to attract females primarily by wing stridulation. This process involves the scraping together of specially modified forewings functioning as sound generators. Bush-crickets and crickets diverged some 240 million years ago, with each lineage developing unique characteristics in sound generator morphology. Bush-crickets (Tettigoniidae) usually have asymmetric wings, and only one wing bears the main sound radiator, the 'mirror'. Properties of this wing cell dictate parameters of frequency and quality in the acoustic signal. In contrast, crickets (Gryllidae), a separate lineage with symmetric wings, use another cell for sound radiation, the harp, with reduced mirrors on both wings playing secondary roles. The grigs (Prophalangopsidae), a relict lineage more closely related to bush-crickets than crickets, have retained a more plesiomorphic wing anatomy. They exhibit symmetrical wings and weakly delimited wing cells including the harp and mirror. This relict group therefore is of major importance to investigate the early evolutionary stages of a critical innovation. This research investigates whether wing biophysics in grigs is more similar to that of bush-crickets or crickets. Using direct evidence from Laser Doppler Vibrometry, this study confirms the mirror cell as an acoustic resonator in the relict species *Cyphoderris monstrosa*. Properties of the mirror in dictating the frequency of the signal are considered in relation to the biomechanics of sound production in bush-crickets. These results further our understanding of the role of wing veins, and the functional cells they delimit, in the evolution of acoustic characters in this diverse group.

### A8.4 QUANTIFYING PULSING PATTERNS IN XENIID CORALS: A FIRST STEP TO COUPLING COLLECTIVE BEHAVIOUR AND FLUID DYNAMICS

TUESDAY 5 JULY, 2016 11:15

JULIA E SAMSON (UNC CHAPEL HILL, UNITED STATES), LAURA A MILLER (UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL, UNITED STATES)

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Corals are mostly stationary organisms. Xeniid corals, however, display a unique behaviour: individual polyps within a colony actively pulse, increasing the local water flux and thus mass transfer (i.e. nutrient and gas exchange). Since cnidarians (corals, jellyfish, anemones, and their relatives) lack a centralized nervous system or integration centre, it is unclear how collective behaviour emerges within a colony. Our assumption is that the many individual polyps follow the same rules when responding to external cues such as flow velocity or shear stress; e.g. pulsing frequency might be related to background flow velocity. In this study, using network theory and neuroscience modelling tools, we will examine whether recurring pulsing patterns can be observed within a colony (i.e. whether the colony functions as a predictable network of polyps). We will also investigate phase differences between pairs of pulsing polyps by analysing video data and generating 2D immersed boundary (IB) models of polyp pairs pulsing at varying phase difference; this will enable us to look at the effect of pulsing phase difference on bulk transport. Additionally, we recorded video data in the lab and the field and manipulated the ambient flow around coral colonies to observe their behavioural response to environments without any flow and environments with steady flows of different average velocities. From these data, we will present preliminary results on the relationship between flow velocity and pulsing frequency.

### A8.5 MORPHOLOGY AND 3D FAST-START ESCAPE PERFORMANCE OF PREGNANT AND VIRGIN LIVE-BEARING FISH

TUESDAY 5 JULY, 2016 11:30

MIKE FLEUREN (WAGENINGEN UNIVERSITY, NETHERLANDS), JOHAN L. VAN LEEUWEN (WAGENINGEN UNIVERSITY, NETHERLANDS), BART J.A. POLLUX (WAGENINGEN UNIVERSITY, NETHERLANDS) ATES)

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A live-bearing mode of reproduction poses several challenges to fish in the family Poeciliidae: the internal development of embryos causes an increase in volume which in turn can potentially reduce a female's swimming performance during pregnancy due to increased drag and decreased flexibility in the abdominal region. We studied the changes in morphology and 3D fast-start escape performance in pregnant and virgin *Poeciliopsis turneri*. Body shape is analysed in 3D by converting outlines from lateral and ventral images into a stack of cross-sections consisting of merged cubic splines (trunk) and superellipses (eyes). Total body volume increased on average 20% during the 8–10 days between parturitions. Maximum width and

height increased approximately 20% and 10% respectively, and the position of maximum width shifted caudally in pregnant fish. The fast-start escape performance was measured in a cubic swimming arena, allowing maximal freedom in escape trajectories, and the stimulus was applied from above. We used three orthogonally placed high-speed video cameras to track body kinematics in 3D. In both pregnant and virgin fish the escape was typically directed downwards. Although maximum velocity was significantly lower in pregnant fish, the vertical velocity component was not. We propose that a more ventral location of the centre of mass could potentially help pregnant fish to maintain equal downwards escape velocities.

## A8.6 INDIVIDUAL AND TOTAL DRAG OF MOTHER AND CALF IN DOLPHIN DRAFTING

TUESDAY 5 JULY, 2016 11:45

MAAKO MIYAKE (TOKAI UNIVERSITY, JAPAN), YOSHINOBU INADA (TOKAI UNIVERSITY, JAPAN), MAMI SAI (TOKAI UNIVERSITY, JAPAN), SHUN TAKAHASHI (TOKAI UNIVERSITY, JAPAN), MAI SAKAI (KINDAI UNIVERSITY, JAPAN), TADAMICHI MORISAKA (TOKAI UNIVERSITY, JAPAN)

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When two dolphins such as mother and calf swim side by side, the calf seldom flaps its tail fin, indicating the calf utilizes the fluid dynamic force between the two bodies as a thrust. This behavior is called 'dolphin drafting'. Our previous study investigated about the dolphin drafting focusing on the fluid dynamic forces acting on the calf and showed that the drag on the calf took the minimum value when the calf was a little behind the mother. In this research, the fluid dynamic forces acting on the mother was investigated under the same condition as the previous study. As a result, the drag of the mother took the maximum value at almost the same position where the calf took the minimum value of the drag. It decreased gradually as the calf moved posteriorly and got lower than the drag measured individually when the calf was around the tail fin of the mother. The total value of the mother and the calf's drag also decreased as the calf moved posteriorly and got lower than the added value of the drag of each individual when the calf was a little behind the position where it took the minimum value of the drag. In conclusion, this research revealed the relationship between the position of the calf and the drag of the mother or the total drag of the mother and the calf.

## A8.7 BEHAVIOURAL ADAPTATIONS OF LOCUSTS UNDER INCREASED MECHANICAL STRESS

TUESDAY 5 JULY, 2016 12:00

JAN-HENNING DIRKS (UNIVERSITY OF APPLIED SCIENCES BREMEN, GERMANY), CHANTAL GÖTTLER (MAX-PLANCK-INSTITUTE FOR INTELLIGENT SYSTEMS STUTTGART, GERMANY)

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The effect of mechanical stress on animal endoskeletons and plant structures is relatively well studied. Interestingly, very little is known about how organisms with exoskeletons react to increased mechanical stress. Previous studies have indicated that mild external stress, such as hypergravity, heat or cold, can have a positive effect on ageing and longevity of some arthropods. Too high stress levels however can induce quiescence behaviour and ultimately increase the risk of starvation. How do different levels of increased mechanical stress affect the biomechanics and behaviour of insects? To answer this question, *Schistocerca gregaria* locusts were raised in an experimental centrifuge setup at 3g and 5g conditions. Our results show that locust kept under control conditions increased their body weight significantly within the first three weeks. Locusts raised under 3g conditions also showed a significant increase in body weight, however a reduced survival rate. However, locusts raised at 5g showed no significant increase of body weight over time, moved significantly less, and a further decreased survival rate. In addition to the behavioural adaptations, our results indicate that raising locusts at increased mechanical stress also affects morphological and biomechanical properties of the exoskeleton. Our results for example show that the density of exoskeletal parts (weight per length tibia) of locusts subject to 5g mechanical load was significantly lower than the leg density of control and 3g locusts. The observed reduction of body weight might affect endocuticular growth, as it is known, that insects use the endocuticle as reserve to overcome starvation periods.

## A8.8 MEASUREMENT OF JUMPING FORCE OF A FRUIT FLY USING A MEMS FORCE PLATE

TUESDAY 5 JULY, 2016 12:15

HIDETOSHI TAKAHASHI (THE UNIVERSITY OF TOKYO, JAPAN), RYU FURUYA (THE UNIVERSITY OF TOKYO, JAPAN), THANH-VINH NGUYEN (THE UNIVERSITY OF TOKYO, JAPAN), TOMOKO YANO (THE UNIVERSITY OF TOKYO, JAPAN), KEI ITO (THE UNIVERSITY OF TOKYO, JAPAN), TOMOYUKI TAKAHATA (THE UNIVERSITY OF TOKYO, JAPAN), KIYOSHI MATSUMOTO (TOYO UNIVERSITY, JAPAN), ISAO SHIMOYAMA (THE UNIVERSITY OF TOKYO, JAPAN)

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Small insects can take-off quickly with an acceleration up to 10G. During taking-off, many insects utilize both jumping force by exerted legs and aerodynamic force by flapping wings simultaneously. However, the contributions of these two forces on the taking-off acceleration are not fully understood.

In this research, we propose a Micro Electro Mechanical Systems (MEMS) force plate to directly detect the jumping force in vertical

direction of a fruit fly (*Drosophila*) during taking-off. When a fruit fly takes off from the plate, the jumping force is measured through the resistance changes of the piezoresistors formed on the force plate.

The mass and taking-off time of fruit flies are approximately 1 mg and 5 ms, respectively. The proposed force plate was designed to achieve a force resolution of less than 1.0  $\mu$ N (tenth part of the weight of a fruit fly). Moreover, the resonant frequency of the plate was designed to be more than 500 Hz to precisely detect the jumping force during the short taking-off time.

The experimental results show that the maximum value of the jumping force reached 150  $\mu$ N, which is approximately ten times larger than the weight of the fruit fly. We also compared the impulse calculated from the measured jumping force and the kinetic momentum obtained from high speed camera images. The result shows that the impulse was equal to 90% of the kinetic momentum, which indicates that the jumping force by legs is the main factor for take-off motion of a fruit fly.

### A8.9 3D-MORPHOLOGY OF INDUCIBLE MORPHOLOGICAL DEFENCES IN *DAPHNIA*

TUESDAY 5 JULY, 2016 12:30

MARTIN HORSTMANN (RUHR UNIVERSITY BOCHUM, GERMANY), PETRA STAMM (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM), ALEXANDER T. TOPHAM (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM), GEORGE W BASSEL (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM), SEBASTIAN KRUPPERT (RUHR UNIVERSITY BOCHUM, GERMANY), JOHN K. COLBOURNE (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM), RALPH TOLLRIAN (RUHR UNIVERSITY BOCHUM, GERMANY), LINDA C. WEISS (RUHR UNIVERSITY BOCHUM, GERMANY)

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Quantitative analysis of shape and form is critical in many biological disciplines as alterations reflect a visible result of e.g. changes in gene expression and physiology. Published 3D-shape capture and render methods produce models with arbitrarily numbered mesh points, preventing a direct comparison of individual point positions derived from different morphotypes. We here introduce a strategy that allows the generation of comparable 3D models, which can also be used for comparative finite element analysis. Specimens are scanned using confocal imaging or alternative strategies conserving information on the third dimension. Subsequently, surface structures are extracted for averaging, comparison and visualisation in Matlab. Via so-called 'casts' sets of statistically comparable point clouds can be acquired. After running a Procrustes-fit, calculation of displacement vectors between vertices of the defended and undefended model are conducted, enabling a coloured display of regions of interest by plotting the vectors' lengths as heat maps in Matlab. We tested our approach with different morphotypes of the pond-dwelling microcrustacean *Daphnia*, which is an excellent model for shape comparisons, since it forms various morphotypes under varying environmental conditions, e.g. predation pressure. With our strategy we are now able to detect significant shape alterations in all spatial dimensions that may alter the handling and biomechanical performance in the mouthparts of respective predators.

### A8.10 HOW PARASITIC WASPS EXPLORE THE UNKNOWN WITH THEIR STEERABLE OVIPOSITORS

WEDNESDAY 6 JULY, 2016 09:00

UROS CERKVENIK (WAGENINGEN UNIVERSITY, NETHERLANDS), BRAM VAN DE STRAAT (WAGENINGEN UNIVERSITY, NETHERLANDS), SANDER W. S. GUSSEKLOO (WAGENINGEN UNIVERSITY, NETHERLANDS), JOHAN L. VAN LEEUWEN (WAGENINGEN UNIVERSITY, NETHERLANDS)

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To lay their eggs, parasitic wasps probe substrates with their slender and steerable ovipositors. The ovipositor consists of three individual elements (valves), one big and two small that are longitudinally connected and can slide along each other. Pro- and retraction of individual valves has been hypothesised to be essential for insertion and steering. Friction along the stationary valves presumably anchors one part of the ovipositor in the substrate and provides lateral support for the moving valve. The ovipositor can be steered in a particular direction by protracting one valve over a greater distance than the others. We tested this hypothesis, and analysed probing mechanisms, performance (i.e. the range and speed of probing) and effects of substrate density on probing. Using two high-speed video cameras, we captured the probing of *Diachasmimorpha longicaudata* (fam. *Braconidae*) in transparent gelatine of two different densities. Wasps thoroughly explore the surrounding substrate through a single puncture hole by creating complex, spatially separated, 3D trajectories with their ovipositors. These are achieved by adjusting the valve kinematics during insertions and by partial retractions, changes in tip orientation, and reinsertions of the ovipositor. A big difference in valve protraction leads to strong curvatures, whereas their equal protraction results in straight paths. A denser gel leads to lower average speeds of insertion and a reduced range of probing. Curvature of the ovipositor decreases the instantaneous speeds of insertion. The substrate dictates probing capabilities which is important for our understanding parasitoid-host interactions and in designing bioinspired minimally invasive steerable needles.

### A8.11 AGE RELATED CHANGES IN THE MECHANICAL PROPERTIES OF INSECT CUTICLE

WEDNESDAY 6 JULY, 2016 09:15

MAEVE O'NEILL (TRINITY COLLEGE DUBLIN, IRELAND), EOIN PARLE (TRINITY COLLEGE DUBLIN, IRELAND), CLODAGH DOOLEY (TRINITY COLLEGE DUBLIN, IRELAND), DAVID TAYLOR (TRINITY COLLEGE DUBLIN, IRELAND)

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We investigated how the mechanical properties of desert locust cuticle (*Schistocerca gregaria*) changed over the course of the insect's adult life. Previous studies showed that 14 days post moult the Young's modulus (E) of the cuticle was 3 GPa. Our results agreed with these but we found that as the insect aged E continued to increase rapidly until about 21 days, before levelling off at approximately 7 GPa for times up to 60 days. The same trend was seen for the failure strength, levelling off at approximately 170

MPa. All mechanical tests were performed via cantilever bending. When we looked at the deposition of the different cuticular layers we discovered that the exocuticle stopped being deposited a few days after moulting, whilst the endocuticle continued to be deposited over the course of the insect's life. This would suggest a decrease in stiffness and strength, contradictory to our results, indicating that an increase in exocuticle thickness is not responsible for the measured changes. We instead propose that water loss is responsible for the change in mechanical properties. Further work is being carried out currently to evaluate water content as a function of age and relate it to E and failure strength.

## A8.12 THE BIOMECHANICS OF INJURY REPAIR IN INSECTS

WEDNESDAY 6 JULY, 2016 09:30

DAVID TAYLOR (TRINITY COLLEGE DUBLIN, IRELAND), EOIN PARLE (TRINITY COLLEGE DUBLIN, IRELAND), JAN-HENNING DIRKS (UNIVERSITY OF BREMEN, GERMANY)

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We carried out the first ever biomechanical study of injury repair in an arthropod. We placed incisions (scalpel cuts) in the tibiae of adult locusts (*Schistocerca gregaria*). At the time of application, the incision reduced the bending strength of the tibia to about one third of its original value. Within three weeks this strength had returned to about two thirds of normal, as a result of deposition of endocuticle underneath the incision, forming a patch. We showed that this deposition was stimulated by the incision: the deposition rate increased by a factor of four. Furthermore, deposition was targeted to the damaged area, implying a process orchestrated by endothelial cells. Computer modelling by finite element analysis suggested that failure of the repaired tibia occurs when the material in the patch reaches its tensile strength. Interestingly, repair only occurred in about half of the subjects: the other half failed to create the endocuticle patch. This may have been because they were too old, or because the gap made by the incision was too wide: further work is ongoing to investigate these aspects.

## A8.13 METACHRONAL LEG COORDINATION AND BODY DYNAMICS IN RAPID RUNNING COCKROACHES

WEDNESDAY 6 JULY, 2016 09:45

TOM WEIHMANN (UNIVERSITY OF COLOGNE, GERMANY), WALTER FEDERLE (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM)

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Alternating tripod leg coordination, i.e. the synchronous activity of the ipsilateral front and hind legs with the contralateral middle legs, is often regarded as the only pattern employed by fast running insects. Observed deviations have rarely been analysed in the context of physical and biomechanical constraints. Here we examine fast level locomotion of speckled cockroaches (*Nauphoeta cinerea*) on two substrates with different nominal asperity sizes of 30  $\mu\text{m}$  and 12  $\mu\text{m}$ ; the latter substrate was markedly slippery for the animals. Steady runs were recorded with a high speed video system and analysed in the horizontal and in the sagittal plane. We measured

the insects' fore-aft, lateral and vertical velocities and accelerations, as well as pitch and yaw. Moreover, kinematic parameters of the legs were examined, such as stride frequency, duty factors and the phase relations between legs. While the phase shift between the ipsilateral middle and hind legs was close to 0.5 and constant over the whole speed range, the shift between ipsilateral front and middle legs decreased from 0.5 at intermediate speeds to 0.32 for fast running. As a consequence, the three legs of each tripod made ground contact successively rather than synchronously, vertical amplitudes of the COM decreased significantly and the increase of the stride frequency levelled off. Moreover, all these changes occurred at lower speeds on the slippery substrate. For the first time the observed changes indicate a change from a symmetric tripod gait to a gallop-like metachronal gait pattern at high running speeds in insects.

## A8.14 INFLUENCE OF INCLINE AND GRANULAR MEDIA ON THE LOCOMOTOR KINEMATICS OF SALAMANDERS

WEDNESDAY 6 JULY, 2016 10:00

KRIJN B MICHEL (ROYAL VET COLLEGE, UNITED KINGDOM), JEFFERY W RANKIN (ROYAL VET COLLEGE, UNITED KINGDOM), LUCY CLARKSON (ROYAL VET COLLEGE, UNITED KINGDOM), ALFREDO G NICIEZA (UNIVERSITY OF OVIEDO, SPAIN), JOHN R HUTCHINSON (ROYAL VET COLLEGE, UNITED KINGDOM)

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Previous studies have proposed that the early origin of tetrapod locomotion is either "hindlimb-driven" or "crutching"-like (i.e., driven by the forelimbs), depending on the taxa. The tail has also been proposed to help improve stability or provide additional body support during locomotion. However, few data are available from extant functional models that can provide a foundation for evaluating the relative contributions of pectoral, pelvic and caudal appendages on terrestrial movement, particularly on inclined substrates that flow in response to intrusion such as mud or sand. In order to quantify the performance of vertebrate appendages on complex media, we compared morphological and kinematic variables of forelimb, hindlimb and tail movement in 20 adult fire salamanders (*Salamandra salamandra*) during locomotion over sand-like and solid surfaces at 0-, 10-, 20- and 30-degree slopes of upward incline. We found an expected decline in maximum locomotion velocity with increased slopes and further decreases in maximum velocity over the sand-like surface. Based on duty factor, the forelimbs have a greater contribution to locomotion on both surfaces. Interestingly, we did not see consistent correlation of tail usage across surface type or slope. Our results support the inference that, despite a clear decline in speed and increase in difficulty of propulsion due to increased slope and substrate flow, the locomotor pattern in *Salamandra salamandra* does not change radically. This may be evidence of a relatively robust neural motor control system in tetrapods, but further biomechanical data and/or tests with models or robots are needed to test this speculation.

## A8.15 MAKING FOOTPRINTS WITHOUT FEET: MODERN TERRESTRIAL LUNGFISH LOCOMOTION CREATES TRACES SIMILAR TO THOSE OF EARLY TETRAPODS

WEDNESDAY 6 JULY, 2016 10:15

PETER L FALKINGHAM (LIVERPOOL JOHN MOORES UNIVERSITY, UNITED KINGDOM), ANGELA M HORNER (CALIFORNIA STATE UNIVERSITY SAN BERNARDINO, UNITED STATES)

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Trackways produced by tetrapods may be comprised of little more than paired circular impressions. Some primarily aquatic vertebrates make brief forays onto land, creating traces as they do. A lack of studies on aquatic trackmakers raises the possibility that such traces may be ignored or misidentified in the fossil record. Although extant fishes are quite distinct from Devonian fishes both morphologically and phylogenetically, several terrestrial Actinopterygian and Sarcopterygian species have been proposed as possible models for ancestral tetrapod locomotion. Although locomotion has been well-studied in some of these taxa, terrestrial trackway production has not. We recorded terrestrial locomotion of a 35 cm African lungfish (*Protopterus annectens*; Dipnoi: Sarcopterygii) on sediment. Terrestrial movement in the lungfish is accomplished by planting the head and then pivoting the trunk. Impressions are formed where the head impacts the substrate, while the body and fins produce few traces. The head leaves a series of alternating left-right impressions, where each impact can appear as two separate semi-circular impressions created by the upper and lower jaws, bearing some similarity to tetrapod traces. Further studies of trackways of extant terrestrial fishes are necessary to understand the behavioural repertoire that may be represented in the fossil record.

## A8.16 RATE-DEPENDENCE OF 'WET' BIOLOGICAL ADHESIVES AND THE FUNCTION OF THE PAD SECRETION IN INSECTS

WEDNESDAY 6 JULY, 2016 11:00

DAVID LABONTE (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), WALTER FEDERLE (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM)

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Many insects use soft adhesive footpads for climbing. The surface contact of these organs is mediated by small volumes of a liquid secretion, which forms thin films in the contact zone. Here, we investigate the role of viscous dissipation by this secretion and the 'bulk' pad cuticle by quantifying the rate-dependence of the adhesive force of individual pads. Adhesion increased with retraction speed, but this effect was independent of the amount of pad secretion present in the contact zone, suggesting that the secretion's viscosity did not play a significant role. Instead, the rate-dependence can be explained by relating the strain energy release rate to the speed of crack propagation, using an established empirical power law. The 'wet' pads' behaviour was akin to that of 'dry' elastomers, with an equilibrium energy release rate close to that of dry van-der-Waals contacts. We suggest that the secretion

mainly serves as a 'release layer', minimising viscous dissipation and thereby allowing rapid detachment during locomotion. In contrast to many commercial adhesives which derive much of their strength from viscous dissipation, we show that the major modulator of adhesive strength in 'wet' biological adhesive pads is friction, exhibiting a much larger effect than retraction speed. Together, these results suggest that 'wet' and 'dry' biological adhesives may be more similar than previously thought.

## A8.17 OPTIMIZING ENERGY STORAGE IN BIOLOGICAL SPRINGS

WEDNESDAY 6 JULY, 2016 11:15

GREGORY P SUTTON (UNIVERSITY OF BRISTOL, UNITED KINGDOM), SHEILA N PATEK (DUKE UNIVERSITY, UNITED STATES), GREGORY S SAWICKI (UNIVERSITY OF NORTH CAROLINA CHAPEL HILL, UNITED STATES), MICHAEL V ROSARIO (BROWN UNIVERSITY, UNITED STATES)

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An open question in biological systems is 'What stiffness maximizes the energy stored in a muscle/spring system?'. Energy storage is the algebraic product of force times distance, which sets up a simple mathematical framework for this question. On the one hand, very compliant springs can be stretched very large distances with small amounts of force. On the other hand, very stiff springs will stretch short distances with large amounts of force. To address this question, I first discuss a simple model system of a muscle isometrically loading a spring. The length-tension property of a muscle provides a limitation on both the force and distance in which a muscle can act; creating a clear optimal stiffness for any spring that this muscle is attached. To test this analysis, it will then be repeated with the biological isometric muscle/spring system within the jumping grasshopper. In the grasshopper, this analysis predicts a spring stiffness of 18 N/mm would maximize the amount of energy stored in the system. The biologically measured spring stiffness is approximately 20 N/mm, within 15% of the theoretical optimal. In more dynamically loaded systems, such as in the bullfrog jump, an isometric analysis, however, is unable to predict the optimal stiffness, but an analysis that considers the muscle force/velocity properties predicts an optimal stiffness of 7.2 N/mm, which is within 10% of the measured stiffness within the system (7.9 N/mm). Consequently, in both isometric and dynamic muscle/spring systems, muscle properties define optimal stiffnesses for the commensurate springs.

## A8.18 BASIC MECHANICS BEHIND STEADY-STATE AND NON-STEADY-STATE MUSCLE CONTRACTIONS

📅 WEDNESDAY 6 JULY, 2016 ⌚ 11:30

👤 MICHAEL GÜNTHER (UNIVERSITÄT STUTTGART, GERMANY), DANIEL F B HAEUFLE (UNIVERSITÄT STUTTGART, GERMANY), SYN SCHMITT (UNIVERSITÄT STUTTGART, GERMANY)

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Four years ago, same meeting, we had exposed a most simple model, consisting of just four mechanical elements, for explaining mechanical power and heat output during concentric steady-state contractions of skeletal muscle. Such reductionist modelling following Ockham's razor helps to make explanatory ideas about real world phenomena transparent and enables challenging, and potentially rejecting, them. Eventually, a question came from the audience, sharp as a razor: 'And what about non-steady-state contractions?'. Only as of recently, we are ready for a stable answer. Here, we show that the basic mechanical structure of a single cross-bridge can explain both early responses to short steps in muscle length and steady-state output. An essential part of this finding is, firstly, that just two model parameters are free to be chosen for fitting the characteristics measured in step-in-length experiments (non-steady-state), whereas the other six reflect literature data, including microscopic geometry and assuming Coulomb repulsion being the elementary drive. Secondly, macroscopic, steady-state characteristics can be derived from exactly the same model, with (i) the force level simply scaled up, (ii) assuming that ATP hydrolysis adds to damping as a second process in steady-state, and (iii) using the contribution of the fibre-internal elasticity to external contraction velocity as one of just two free parameters to fit the steady-state characteristics. We conclude that the suggested model structure may constitute an irreducible mechanical core based on which any contribution of active muscle to interactions with its environment can be examined.

## A8.19 FUNCTIONAL MORPHOLOGY, BIOMECHANICS AND FINITE ELEMENT SIMULATION OF *SCHEFFLERA* RAMIFICATIONS FOR BIOMIMETIC APPLICATIONS IN CIVIL ENGINEERING

📅 WEDNESDAY 6 JULY, 2016 ⌚ 13:45

👤 KATHARINA BUNK (PLANT BIOMECHANICS GROUP AND BOTANIC GARDEN UNIVERSITY OF FREIBURG, GERMANY), FLORIAN JONAS (INSTITUTE OF BUILDING STRUCTURES AND STRUCTURAL DESIGN UNIVERSITY OF STUTTGART, GERMANY), JAN KNIPPERS (INSTITUTE OF BUILDING STRUCTURES AND STRUCTURAL DESIGN UNIVERSITY OF STUTTGART, GERMANY), THOMAS SPECK (PLANT BIOMECHANICS GROUP AND BOTANIC GARDEN UNIVERSITY OF FREIBURG, GERMANY), TOM MASSELER (PLANT BIOMECHANICS GROUP AND BOTANIC GARDEN UNIVERSITY OF FREIBURG, GERMANY)

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The ramification of *Schefflera arboricola* exhibits a conspicuous branching morphology with several finger-like side branches that originate from the stem and merge distally in the branch. Therefore this species has been selected as an interesting biological concept generator for the biomimetic development of nodal elements in branched technical pillars for building constructions. The branching morphology and the internal arrangement of vascular bundles as mechanically relevant tissue have been analyzed via serial thin sectioning and  $\mu$ CT-scanning. This allows for reconstructing the internal course and arrangement of vascular bundles in a three-dimensional model of the stem-branch-attachment and for Finite Element (FE) simulations of realistic stress distributions and deformation of the biological ramification. These simulations require high resolution 3D information of the branching morphology and anatomy from  $\mu$ CT and/or 3D-laser scans. A detailed image acquisition, image post-processing and transfer into a closed surface mesh are major challenges prior to the simulation process. Results of the FE-simulations are compared with and validated by the results of biomechanical tests of *Schefflera* stem-branch-attachments. The combination of mechanical testing and simulation shall serve for a deepened understanding of the mechanical relevance of the finger-like branching morphology in *Schefflera*. This process allows for abstracting the most promising structures and functional principles for developing biomimetic branched-fiber-reinforced polymer tubes filled with concrete that will be used for optimizing branched pillars for application in architecture.

## A8.20 BIOMECHANICAL ANALYSIS OF THE ENDOCARP OF *COCOS NUCIFERA*

📅 WEDNESDAY 6 JULY, 2016 ⌚ 14:00

👤 STEFANIE SCHMIER (UNIVERSITY OF FREIBURG PLANT BIOMECHANICS GROUP BOTANIC GARDEN FIT, GERMANY), DOMINIK OTTERS (UNIVERSITY OF FREIBURG PLANT BIOMECHANICS GROUP BOTANIC GARDEN, GERMANY), GEORG BAUER (UNIVERSITY OF FREIBURG PLANT BIOMECHANICS GROUP BOTANIC GARDEN FIT, GERMANY), MARC THIELEN (UNIVERSITY OF FREIBURG PLANT BIOMECHANICS GROUP BOTANIC GARDEN FMF, GERMANY), THOMAS SPECK (UNIVERSITY OF FREIBURG PLANT BIOMECHANICS GROUP BOTANIC GARDEN FIT FMF, GERMANY)

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Ripe fruits of the coconut palm (*Cocos nucifera*) weigh up to 3.7 kg and the palms reach heights of up to 30 m. Therefore, to assure the germination of the embryo the fruit has to withstand severe impacts of up to 1 kJ when dropping onto the ground. In addition the coconut fruits (which botanically represent drupes) remain germinable even after several months of dispersal in seawater. These properties are ensured by the triple-layered fruit wall of the drupe protecting the seed, which consists of a leathery exocarp, a fibrous mesocarp and a tough endocarp.

Currently under investigation are the mechanical properties of the endocarp of the coconut as to fracture behaviour and to crack propagation in this tough structural material. First results with an impact pendulum reveal differing mechanical properties depending on the cutting direction of the tested samples from the endocarp. These results suggest a pronounced anisotropy of this structural material reflecting anatomical differences depending on the cutting direction in the endocarp such as number of vascular bundles, sclereid orientation or dimensions. Identifying the linkage of anatomical structures on different hierarchical levels of the endocarp with the mechanical behaviour during impact is of special interest for the transfer into bioinspired technical applications. Those applications can for example be found in the field of building constructions, where a combination of light weight structuring with high energy dissipation capacity and puncture resistance is of increasing interest to protect sensible buildings e.g. against earthquakes, rock fall and other natural or manmade hazards.

## A8.21 TRAP DIVERSITY AND EVOLUTION IN CARNIVOROUS BLADDERWORTS (*UTRICULARIA*, *LENTIBULARIACEAE*)

📅 WEDNESDAY 6 JULY, 2016 ⌚ 14:15

👤 ANNA S WESTERMEIER (PLANT BIOMECHANICS GROUP BOTANIC GARDEN FREIBURG, GERMANY), THOMAS SPECK (PLANT BIOMECHANICS GROUP BOTANIC GARDEN FREIBURG, GERMANY), SIMON POPPINGA (PLANT BIOMECHANICS GROUP BOTANIC GARDEN FREIBURG, GERMANY)

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One of the fastest plant motions known is the prey capture by suction traps of aquatic carnivorous bladderworts (*Utricularia* spp.). Trap functioning relies on a mechanical instability mechanism of the rapidly opening and closing trapdoor. However, knowledge on the trapdoor motion in species of other life-forms (i.e. terrestrial), representing the most species-rich group of the genus, and the respective functional adaptations to the different habitats is scarce. We studied non-aquatic bladderwort species from various generic sections and examined possible linkages between life-forms, trapping mechanisms, functional trap morphology and phylogeny using high-speed video analyses, microscopy techniques (LM and SEM) and particle image velocimetry. In our study we show that not only differences in trap entrance and door architecture exist but also in trapdoor motion, and we could classify three main types of trapdoor movement (and several subtypes). Respective insights into fluid dynamics during suction could also be gained. Our results were mapped onto a phylogenetic reconstruction of the genus and we postulate that the phylogenetic lineages also represent lineages of specific structural-functional adaptation. Finally, potential scenarios of trap evolution and species radiation are discussed showing that *Utricularia* may be regarded an exquisite case of adaptation at various structural and functional levels to diverse habitats.

## A8.22 OSCILLATIONS OF TREES CAUSED BY DIFFERENT TYPES OF EXCITATION: AN ANALYSIS OF EXPERIMENTAL RESULTS OBTAINED BY USING A 3D MOTION CAPTURE SYSTEM

WEDNESDAY 6 JULY, 2016 14:30

IVANA KOVACIC (UNIVERSITY OF NOVI SAD FACULTY OF TECHNICAL SCIENCES CENTRE OF EXCELLENCE FOR VIBRO-ACOUSTIC SYSTEMS, SERBIA AND MONTENEGRO), MIODRAG ZUKOVIC (UNIVERSITY OF NOVI SAD FACULTY OF TECHNICAL SCIENCES CENTRE OF EXCELLENCE FOR VIBRO-ACOUSTIC SYSTEMS, SERBIA AND MONTENEGRO), DRAGI RADOMIROVIC (UNIVERSITY OF NOVI SAD FACULTY OF AGRICULTURE, SERBIA AND MONTENEGRO), PAVEL BENKA (UNIVERSITY OF NOVI SAD FACULTY OF AGRICULTURE, SERBIA AND MONTENEGRO), MILUTIN NIKOLIC (UNIVERSITY OF NOVI SAD FACULTY OF TECHNICAL SCIENCES CHAIR OF MECHATRONICS ROBOTICS AND AUTOMATION, SERBIA AND MONTENEGRO)

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Experimental investigations of the dynamic response of potted trees were carried out by using the Vicon 3D motion capture system, which is a leading state-of-the-art infrared marker-tracking system. Reflective markers were arranged along the trunk of a young trunk-dominated tree (*Aesculus hippocastanum*) and along the trunk and branches of a young branched tree (*Cercis siliquastrum*). They are subject to various excitations: static, non-stationary fluid flow, harmonic and non-harmonic base excitation. Dynamic responses of both trees in leaves and without leaves were recorded. In addition, the experiments were repeated with the branched tree whose branches of higher hierarchy were gradually removed. The 3D spatial displacements were then imported into a symbolic software package and the corresponding time-history diagram and trajectories of the markers plotted and further analysed in a time and frequency domain. Numerical and semi-analytical methodologies were developed to determine basic oscillatory characteristics: principle stiffness axes, frequencies of vibrations, as well as viscous damping ratios, which stem from aerodynamical and internal (material) damping. The importance of principle stiffness axes and the frequencies associated with these axes is emphasized, as they have not been considered in Experimental Biology so far.

## A8.23 HOW WATER AVAILABILITY INFLUENCES MORPHOLOGICAL AND BIOMECHANICAL PROPERTIES IN DESICCATION-TOLERANT AND DESICCATION-INTOLERANT HERBACEOUS PLANTS

WEDNESDAY 6 JULY, 2016 14:45

TIM KAMPOWSKI (PLANT BIOMECHANICS GROUP UNIVERSITY OF FREIBURG, GERMANY), MAX MYLO (PLANT BIOMECHANICS GROUP UNIVERSITY OF FREIBURG, GERMANY), SIMON POPPINGA (PLANT BIOMECHANICS GROUP UNIVERSITY OF FREIBURG, GERMANY), THOMAS SPECK (PLANT BIOMECHANICS GROUP UNIVERSITY OF FREIBURG, GERMANY)

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In herbaceous plants, changes in water availability do not only strongly influence metabolic processes, but also cause significant alterations of morphological characteristics and biomechanical properties. Only few plant species have evolved both physiological as well as structural adaptations allowing for survival under extreme drought conditions for up to several months. Such resurrection plants are able to fully recover from a relative water content below 10%, hereby regaining normal shape, functionality and stability, often within a short amount of time upon rehydration. In our experiments, we analysed morphological as well as biomechanical characteristics of closely related desiccation-tolerant *Ramonda myconi* and desiccation-intolerant *Monophyllaea horsfieldii* (both herbaceous members of the family Gesneriaceae) in the context of dehydration-rehydration experiments (DREs). On a macroscopic scale, we investigated the influences of internal cell and tissue pressure variations on the shapes and the overall adaptive mechanical performance of the plants. Furthermore, tissue arrangements as well as cellular and cell wall structures of *Monophyllaea* and *Ramonda* have been comparatively analysed to detect general functional morphological and biomechanical principles on a microscopic scale. Ultimately, in the context of a joint research project among biologists, chemists and material scientists, our findings are planned to be implemented in novel bio-inspired polymer systems based on adaptive non-isocyanate polyurethane networks (NIPU) developed from renewable resources in green chemistry manufacturing processes.

## A8.24 DO FUNGI RELEASE MECHANICAL DORMANCY CONFERRED BY THE SEED COVERINGS IN *LEPIDIUM DIDYMUM*?

WEDNESDAY 6 JULY, 2016 15:00

TINA LH STEINBRECHER (ROYAL HOLLOWAY UNIVERSITY LONDON, UNITED KINGDOM), KATJA SPERBER (UNIVERSITY OF OSNABRUECK, GERMANY), JAMES E HOURSTON (ROYAL HOLLOWAY UNIVERSITY LONDON, UNITED KINGDOM), KAI GRAEBER (ROYAL HOLLOWAY UNIVERSITY LONDON, UNITED KINGDOM), KLAUS MUMMENHOFF (UNIVERSITY OF OSNABRUECK, GERMANY), GERHARD LEUBNER (ROYAL HOLLOWAY UNIVERSITY LONDON, UNITED KINGDOM)

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Plant roots and fungi frequently exist in complex mutualisms with one another and these mutualisms commonly involve the exchange of nutrients between partners, however what is less common is mutualisms between plant diaspores and saprophytic fungi. In this study we provide evidence that fungal-mediated erosion of a pericarp (fruit coat) tissue layer is a targeted process, guided by plant tissue architecture and anticipated by the plant to regulate germination timing. Seed and fruit coats can act as a mechanical barrier to prevent or delay germination. We found that in *Lepidium didymum* (Brassicaceae) the lignified pericarp is colonised by saprophytic fungi which play a key role in germination timing. Puncture-force measurements demonstrate that the pericarp is selectively weakened by the fungi at the micropylar end to allow penetration of the radicle. The *L. didymum* pericarp acts as a mechanical barrier, but it does not restrict water uptake or gas exchange. We identified a specific anatomical region of less lignified cells within the endocarp, representing a preformed breaking zone which is degraded by fungal hyphae. As a consequence, the fungal colonisation of fruits leads to a much faster onset and higher maximum germination as it effectively breaks this pericarp-imposed dormancy.

## A8.25 AERODYNAMIC LOADS OF A BOOBOOK OWL THROUGH PIV MEASUREMENTS

WEDNESDAY 6 JULY, 2016 15:45

ROI GURKA (COASTAL CAROLINA UNIVERSITY, UNITED STATES), HADAR BEN-GIDA (TECHNION, ISRAEL), DANIEL WEIHS (TECHNION, ISRAEL)

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The mechanisms of silent flight of owls have been the subject of scientific interest for many decades and a source of inspiration in the context of reducing flight noise. Over millions of years of evolution, owls have produced many specialized configurations to reduce the aerodynamic noise, which is found to be essential for successful hunting of potential prey. Here, we estimate the aerodynamic loads: drag and lift of a freely flying owl. We study the unique mode of flight of a boobook owl (*Ninox boobook*) a mid-sized owl, which has the feature of stealth flight during both gliding and flapping flight. The owl was flown in a hypobaric avian wind tunnel at its comfort speed for various flight modes. The wake velocity field was sampled using long duration high speed PIV whilst the wing's kinematic were imaged using high-speed video simultaneously

with the PIV. The time series velocity maps acquired during several consecutive wingbeat cycles enabled us to estimate the lift and drag obtained during flight. Specifically, we have calculated the unsteady aerodynamic loads, which resulted from the flapping motion over the wingbeat cycle. Comparison of the unsteady aerodynamic loads is made with other passerines such as starling and sandpiper; individuals of both of which flew in the same wind tunnel under similar flow conditions. Differences between the birds' wakes are presented and connected to the owl's special acoustic capabilities. Estimating drag and lift may shed light on the energy consumption during its unique stealthy flight mode.

## A8.26 EVOLUTION OF THE STOOP OF FALCONS IN A MODEL OF BIRD FLIGHT

WEDNESDAY 6 JULY, 2016 16:00

ROBIN MILLS (UNIVERSITY OF GRONINGEN, NETHERLANDS), HANNO HILDENBRANDT (UNIVERSITY OF GRONINGEN, NETHERLANDS), GRAHAM K. TAYLOR (OXFORD UNIVERSITY, UNITED KINGDOM), CHARLOTTE K. HEMELRIJK (UNIVERSITY OF GRONINGEN, NETHERLANDS)

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The Peregrine falcon *Falco peregrinus* intercepts its prey in a fast-speed, controlled dive from a high altitude, called a stoop. This stoop has fascinated both laymen and researchers because of its enormous speeds of over 320 kmh<sup>-1</sup>. In order to explain why Peregrine falcons adopt this hunting strategy, we have built a bird-flight simulator. In this simulator, birds are bound to approximated aerodynamics and flap and glide to maneuver through the air. Their flight performance (e.g. top speed, turning speed, agility, acceleration) is dependent upon their morphology. The interception strategy or guidance algorithm (namely proportional navigation) of predatory model-birds is validated by empirical experiments on hunting Peregrine falcons. In order to gain speed in dives, model-birds retract their wings appropriately. We use this model to gain understanding about the stoop in two ways. First, we analyze the model equations to understand the advantages and disadvantages of a stoop with respect to steering, guidance, agility and maneuverability. Second, we simulate hunts of Peregrine falcons on four different prey species. We let the prey fly in several ways, ranging from linear to highly erratic. For each prey species and prey-movement type, we apply evolutionary algorithms to evolve the hunting strategy of the falcon. Interestingly, stoops evolve as (precision) optimal strategy for hunting on all prey as long as they maneuver slightly or strongly. We interpret these results using our aerodynamic analyses: stoops not only increase the falcon's forward speed, agility, maneuverability and acceleration, it also provides advantages for steering and guidance.

## A8.27 POWER OF THE WINGBEAT: EFFECTS OF FLAPPING WINGS IN VERTEBRATE FORWARD FLIGHT

WEDNESDAY 6 JULY, 2016 16:15

MARCO KLEIN HEERENBRINK (LUND UNIVERSITY, SWEDEN), L. C. JOHANSSON (LUND UNIVERSITY, SWEDEN), A. HEDENSTRÖM (LUND UNIVERSITY, SWEDEN)

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Flight can be an expensive mode of transport, and for many flying animals it takes a significant part of their energy budget. Often fixed wing aircraft models are used to estimate flight cost, where energetic cost as a function of flight speed is expressed in terms of weight, wingspan, wing area and body area, with specific aerodynamic details represented by coefficients. Animals flap their wings to produce thrust, affecting these aerodynamic coefficients in a way that is distinctly different from aeroplanes and helicopters. Particularly the effects on induced power have received little attention. We developed a model that takes into account the effects of the reciprocating wing motion, by using aerodynamic coefficients that are explicitly formulated in terms of thrust requirement, wingbeat frequency and stroke-plane angle, while implicitly optimizing wingbeat amplitude for minimum induced power. The model indicates that previously assumed values for the induced power factor are typically underestimated. We have found empirical support for the predicted effects on induced flow from wake measurements behind a jackdaw (*Corvus monedula*) flying in a wind tunnel, where we measured induced drag factors close to the predicted minimum values of 1.5 at low speeds. At higher speeds the factor increased up to 2.5, which is in sharp contrast to the range of 0.9 to 1.2 commonly found in literature, suggesting flying animals are not as efficient as we once thought.

## A8.28 WING DAMAGE CONTROL IN FLYING FRUIT FLIES

WEDNESDAY 6 JULY, 2016 16:30

FLORIAN T MUIJRES (WAGENINGEN UNIVERSITY, NETHERLANDS), NICOLE A IWASAKI (UNIVERSITY OF WASHINGTON, UNITED STATES), MICHAEL J ELZINGA (UNIVERSITY OF WASHINGTON, UNITED STATES), MICHAEL H DICKINSON (CALIFORNIA INSTITUTE OF TECHNOLOGY, UNITED STATES)

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The wings of animals are susceptible to damage, which can occur through general wear or specific events such as collisions or predator attack. Unlike birds and bats that possess dedicated wing damage repair mechanisms such as molt, insects cannot repair their wings and thus need to cope with the detrimental effects of wing damage for the rest of their life. The most direct consequence of wing damage is the alteration of aerodynamic forces and moments due to the loss of wing area, and this might reduce flight performance and agility. By combining high-speed videography measurements on flying fruit flies with experimentally induced wing damage with physical and computational aerodynamics modelling, we determined that the effect of wing damage is on aerodynamic forces and torques, and how fruit flies adjust their wingbeat kinematics to compensate for these detrimental aerodynamic effects. Our results show that unilateral wing damage primarily reduces weight support and causes a roll torque, that if not controlled for would make the fly spin out of control. Fruit flies compensate for these two aerodynamic effects of wing damage by adjusting their kinematics in a modular fashion: to maintain weight support a fruit fly increases wingbeat frequency, and to negate the damage-induced roll torque the animal adjusts the wingbeat pattern of both the intact and damaged wing. Using the robotic and computational aerodynamic models we identified the aerodynamic mechanisms responsible for wing damage control. The study also allowed us to propose a simple bio-inspired algorithm for controlling asymmetric wing damage.

## A8.29 ROTATIONAL STEREO-VIDEOGRAPHY (RSV): A FIELD METHOD FOR 3D TRACKING OF FLYING ANIMALS, IN LARGE AIR VOLUMES.

WEDNESDAY 6 JULY, 2016 16:45

EMMANUEL DE MARGERIE (CNRS, FRANCE), CÉCILE PICHOT (RENNES 1 UNIVERSITY, FRANCE), MANON SIMONNEAU (RENNES 1 UNIVERSITY, FRANCE), JEAN-PIERRE CAUDAL (RENNES 1 UNIVERSITY, FRANCE), CÉCILIA HOUELIER (RENNES 1 UNIVERSITY, FRANCE), SOPHIE LUMINEAU (RENNES 1 UNIVERSITY, FRANCE)

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We present RSV, an optical method for reconstructing flight paths, based on stereo-videography and aiming-angle recording. A single filming device is used, combining a single camera and telephoto lens, a set of mirrors and two rotary encoders. The operator aims at the flying animal, and actively tracks it by rotating the device, keeping the animal within the camera's field of view. For each video frame, the animal can be positioned, within a spherical volume of interest

(VOI) centered on the device. The VOI radius depends on the desirable positional uncertainty (measured as the random error, i.e. the 3D position SD), and in turn constrains the maximal tracking duration. We show that short flight bouts of a few seconds, appropriate for flight kinematics analysis ( $SD < 0.1$  m) can be measured within a radius of about 50 m from the observer. Longer flight paths, up to a few minutes long, allowing spatial behaviour investigation ( $SD < 1$  m), can be recorded within about 200 m. At 500 m from the device, RSV approaches GPS-like uncertainty (i.e.  $SD \approx 5-10$  m). We share example tracks recorded at various ranges, including unpublished prolonged flight tracks of common swifts (*Apus apus*). We discuss the strengths and limitations of RSV compared to other local flight tracking techniques (static multi-camera videography, ornithodolite, etc.). We also point out the potential complementarity of RSV tracking at the local scale, with GPS tracking at the global scale, to better understand spatial behaviour processes, in a movement ecology perspective.

### A8.30 PINE CONE SEED SCALES AS ROLE MODELS FOR ADAPTIVE FLAPS IN ARCHITECTURE

WEDNESDAY 6 JULY, 2016 POSTER SESSION

SIMON POPPINGA (PLANT BIOMECHANICS GROUP FREIBURG, GERMANY), THOMAS SPECK (PLANT BIOMECHANICS GROUP FREIBURG, GERMANY)

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Pine cone seed scales bend as passive reactions to changing air humidity which is based on the different swelling and shrinking properties of the involved tissues. A highly swellable sclereid layer functions as the actuating element, whereas a much less swellable sclerenchymatous tissue functions as a resistance element which dictates the bending. This project among biologists, material scientists/chemists and architects covers basic investigation of this biological principle and its application and transfer into a technical solution (humidity-driven flaps for architecture). We comparatively analyse the biomechanics, the general structural setup and the functional morphology of seed scales from various Pine species. Computational modelling and 3D printing of hygroscopic copolyester with embedded cellulose fibres allows for technical implementation of the movement principles into autonomous flaps with tailored sensitivity and response. Here with it is also possible to produce technical flaps executing complex consecutive motions steps as observed in natural scales.

### A8.31 ACOUSTIC COMMUNICATION: BODY SIZE VERSUS FREQUENCY TUNING AS A BALANCING ACT

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Acoustic communication signals, both their production and reception, are intertwined with all aspects of an animal's biology. An intuitive example is the inverse relationship between maximum signaling frequency and body size; which is well supported across a wide range of animal taxa. But this same relationship, between maximum frequency and body size, also occurs for acoustic sense organs, however the relationship is a bit more complex. The maximum and resonant frequencies of the tympanic membrane are affected by its size, shape, and thickness; which in combination can help to compensate for a wider range of receiver body sizes and frequency ranges as required by life history. In the bush cricket species complex *Ephippiger* a large variety of body sizes can be found within and across multiple populations throughout its range. Despite body size differences, frequency components of male mating calls are quite similar across its range and thus, the hearing organs should also be similarly tuned. Individuals from four different populations in France were exposed to broadband acoustic chirps and the motion of their tympanic membranes measured using a 3D laser Doppler vibrometer. While signal frequency components are not as important for mate choice they do play significant roles in species recognition. Our initial results show that the response of the tympanic membrane is sometimes in agreement with the inverse relationship between frequency and body size; suggesting that at some body sizes a frequency shift of the tympanic membrane's response may be a balancing act too difficult to prevent.

### A8.32 THE EFFECT OF FENCE WIDTH ON PEAK VERTICAL FORELIMB LANDING FORCES IN JUMPING DOGS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

TIMOTHY WHITE (HARTPURY COLLEGE, UNITED KINGDOM), ALISON P WILLS (HARTPURY COLLEGE, UNITED KINGDOM)

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Canine agility is a sport rapidly increasing in popularity, but studies investigating the kinetics of jumping in dogs are limited. Previous research has quantified the relationship between the height of the jump and peak limb landing forces, and has examined the changes in kinematic parameters with increasing jump width. In this study,  $n=8$  Labrador Retrievers with limited agility experience jumped a standard spread jump at widths of 45 cm, 55 cm and 65 cm. Widths were based on the differing maximum jump widths recommended by different international organisations. All dogs completed three valid trials at each width. A forceplate was used to register the peak forelimb landing forces experienced. Data were analysed via a one-way repeated measures ANOVA to test for significant differences in peak limb forces between the three jump widths. There was no significant difference in peak vertical forelimb landing force between the three jump widths tested ( $p=0.207$ ). There was also no

significant difference detected in peak mediolateral ( $p=0.722$ ) and craniocaudal ( $p=0.628$ ) landing forces. Data gained indicated that despite the existence of varying regulations regarding maximum fence width, this does not have a significant impact on the peak forelimb landing forces experienced by jumping dogs. However, this experiment was conducted on the straight and may not be fully representative of an agility course which involves sharp cornering and jump landings that occur on a bend. Fence height and width may not be the only factor that determines peak forelimb landing forces and this warrants further investigation.

### A8.33 THE EFFECT OF HYDROTHERAPY ON THE RANGE OF MOTION OF DOGS DIAGNOSED WITH ELBOW DYSPLASIA

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Canine elbow dysplasia is a debilitating condition that is the most common cause of forelimb lameness in dogs, but the aetiology of this condition is not fully understood. Canine hydrotherapy is a therapeutic approach rapidly increasing in popularity for the treatment of range of musculoskeletal pathologies. In this study, kinematic analysis was used to assess the effect of a customised hydrotherapy session on the range of motion, stride frequency and stride length of a group of clinically sound Labradors ( $n=6$ ) and Labradors diagnosed with bilateral elbow dysplasia ( $n=6$ ). Reflective kinematic markers were attached to bony anatomical landmarks and dogs were recorded walking at their preferred speed on a treadmill before and after a hydrotherapy session. Range of motion, stride length and stride frequency were calculated for both forelimbs. Data were analysed via a robust mixed ANOVA to assess the effect of hydrotherapy on the kinematic parameters of both groups. Range of motion was significantly higher in the control dogs ( $p<0.05$ ), but hydrotherapy significantly increased the range of motion of the forelimbs of both groups ( $p<0.05$ ), with pathological dogs improving significantly more than the healthy group ( $p<0.05$ ). Hydrotherapy also significantly increased stride frequency ( $p<0.05$ ), and stride length ( $p<0.01$ ) of all dogs, but significant differences were not seen between the two groups. This finding indicates the potential of canine hydrotherapy as a therapeutic tool for the rehabilitation and treatment of dogs with musculoskeletal pathologies. Furthermore, results indicate that hydrotherapy may be advantageous in improving the gait and movement of clinically healthy dogs.

### A8.34 KINEMATIC ANALYSIS OF THE GAIT OF PEDIGREE WORKING AND SHOW DOGS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Showing dogs is a popular discipline worldwide with animals from a range of backgrounds participating. Working dogs may be required to perform more physical work and training than dogs kept purely for showing and it is hypothesised that this may result in changes to their gait and musculature that may be disadvantageous within the showing ring. The aim of this study was to identify whether the gait of the pedigree working dog differs from that of a pedigree show dog of the same breed. Kinematic data were recorded from  $n=15$  clinically sound Belgian Shepherd dogs ( $n=8$  working;  $n=7$  showing). Reflective markers were placed at defined anatomical landmarks and dogs were filmed moving at walk, trot and gallop. Stride parameters and range of motion were analysed and compared for the two groups using the Mann-Whitney U test for non-parametric unrelated samples. There was no significant difference in range of motion between the two groups. At walk, the show dogs had a longer stride time ( $p<0.001$ ) and lower stride frequency ( $p<0.001$ ) than the working group. Stride length was not significantly different at walk and trot, but was longer in the working group at gallop ( $p<0.01$ ). No differences in stride time or frequency were detected at trot but at gallop, stride time was longer in the show dogs ( $p<0.05$ ) and stride frequency was lower ( $p<0.05$ ). In conclusion, some kinematic differences were observed between working and show dogs but whether these would be detectable by a show judge requires further research.

### A8.35 WALKING OR HOPPING? EVOLUTIONARY TRENDS IN TERRESTRIAL LOCOMOTION OF NEOTROPICAL BIRDS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Birds can use different types of gaits to move on the ground, as walking, hopping, and running. However, it remains unclear what drives the preference for one or the other gait during terrestrial locomotion. Even if an increase of speed can explain the preference for running, the two other gaits do not necessarily occur with a clear pattern at lower speeds among the diversity of birds. Ecological parameters, such as the type of habitat or the size of the bird can explain why they perform whether hopping, walking, or both alternatively, in addition, the distribution in the phylogeny can be invoked. To explore this question, we performed a morphological analysis on modern birds among a wide range of Neotropical birds in a phylogenetical context. We performed dissections of 23 muscles of the hindlimbs and measured the average fibre lengths for each muscle, as well as other myological parameters. We also used computer tomography to describe the osteology of their pelvis and the three long bones of the hindlimb.

To quantify the morphological differences between the 22 studied species a geometric morphometrics analysis was carried out using independent contrasts. In this context, we tented to reconstruct the ancestral character state and propose functional hypotheses to understand the evolution of hopping and walking in Neotropical birds.

### A8.36 DYNAMICS OF THE BEAK DURING SINGING IN FINCHES

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Studies of Darwin's finches have provided some of science's most compelling examples of how natural selection can drive phenotypic change. It was recently discovered that species specialised to feed on hard seeds have a decreased ability to conduct rapid changes in beak gape during singing. In turn, this limits their performance in producing dynamically complex songs. As songs of Darwin's finches are used in species recognition and mate choice, the observed trade-off between force and velocity of the beak may have had a direct influence on interspecies mating dynamics, probabilities of hybridization, and ultimately the process of speciation. However, it is unknown what causes this biomechanical trade-off. There are several candidates, such as the inertial properties of the skeletal elements involved, the gearing from the muscles to the upper and lower beak via joints and levers, or the size, orientation and architecture of the different jaw muscles involved. The goal of this study is to identify the biomechanical basis of this trade-off via dynamic, multi-body modelling based on a motion analysis of the beak of a species that closely resembles the Darwin's finches (Java finch), and a unique, existing database of 3D morphology of the head of the Java finch and several Darwin's finches.

### A8.37 A NEW APPROACH OF MICROCT FOR RESOLVING THE ANATOMY OF THE MUSCULOSKELETAL SYSTEM OF THE AVIAN WING

WEDNESDAY 6 JULY, 2016 POSTER SESSION

FERNANDA BRIBIESCA CONTRERAS (FACULTY OF LIFE SCIENCES, UNIVERSITY OF MANCHESTER, UNITED KINGDOM), WILLIAM I. SELLERS (FACULTY OF LIFE SCIENCES, UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

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The evolution of a feathered forelimb for flying has been a crucial factor in the success of birds. Understanding the form-function relationship of the musculoskeletal system of the wing will undoubtedly shed further light upon avian evolution and origin of flight. Previous studies have mostly focused on the external anatomy of the wing and less attention has been paid to the flight musculature, especially the more distal muscles in the hand due to their small size and complex 3D arrangement. Anatomical descriptions of the avian myology are available, however, for the

wing muscles the information is still very incomplete. Despite the widespread use of gross dissections for studying animal anatomy, it is a destructive technique that can be very difficult for small specimens. Contrast-enhanced microCT has proved to be a suitable tool for soft tissue visualization. The aim of this project is to assess the ability of contrast-enhanced microCT to reconstruct a 3D model of the musculoskeletal system of the bird wing. A 3% iodine-based buffered formalin solution with a two-weeks staining period was used for soft tissue visualisation of a Sparrow Hawk (*Accipiter nisus*) wing and conclude that this is an effective technique for studying the internal anatomy of the avian forelimb. Contrast-enhanced microCT can produce 3D images of the wing musculature of birds, including the smaller muscles in the hand, and provides a non-destructive way for muscle architecture quantification. Furthermore, a 3D reconstruction of the musculoskeletal system of the Sparrowhawk wing is presented.

### A8.38 AERODYNAMIC CHARACTERISTICS OF A SEABIRD GULL-WING

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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The wing of seabird flying long distance with a long duration has a characteristic shape. It has a high aspect ratio planform and an inverted W-shaped front shape. This type of wing is called 'gull wing' because its representative is a sea gull. In this study, wind tunnel tests were conducted using three types of gull wing models with different front shapes such as a flat, an inverted W-shaped, and an inverted U-shaped wings with a same planform to clarify the relationship between the front shape and the aerodynamic performance. In consequence, the inverted U-shaped wing had the largest lift to drag ratio (L/D) followed by the inverted W-shaped wing. The flat type had the smallest L/D. Regarding the stability, all types had a good static stability around a pitching and a rolling axes but negative stability around a yaw axis. Good pitching and rolling stability might be caused by a sweep-back of the wing tip and the negative yawing stability might be caused by the lack of vertical wing. Thus the basic characteristics of gull wing were clarified.

### A8.39 NUMERICAL ANALYSIS OF THE EFFECT OF CALF'S SIZE ON THE DOLPHIN DRAFTING BY USING OPENFOAM

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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This research investigates about the dolphin drafting of mother and calf. The fluid dynamic interaction between the mother and the calf was analyzed for various sizes and various positions of the calf using Computational Fluid Dynamics (CFD). As a result, it was clarified that some of the parameters depended on the size of the calf and others did not. When the calf took the maximum thrust, the ratio of the longitudinal position of the calf to the size of the calf was almost constant regardless of the size of the calf. The total drag of the mother and the calf decreased as the calf moved posteriorly and became lower than the added value of the drag of each individual regardless of the size of the calf. On the other hand, both the thrust of the calf and the drag of the mother increased as the size of the calf increased. In consequence, the detail of the effect of the calf's size on the dolphin drafting was clarified.

### A8.40 HOW DO BIRDS STAND UP?

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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The sit-to-stand transition (STST) is a movement widely used by terrestrial vertebrates. However, very little research exists on the STST mechanics of nonhuman animals. One key element of the STST is that animals must overcome gravitational constraints to lift the body centre of mass. Because gravitational constraints have increasing influence at larger body sizes, they likely influence the technique that an animal uses to stand. Our prior work found that – despite wide variations in size (0.2–124Kg) – round-running birds start from similar postures during STST. Here we hypothesize that larger birds increase their joint range of motion (ROM) during STST to obtain more upright standing limb postures. To test our hypothesis, marker data were collected via motion capture from five pheasants (0.99Kg) and two emus (24Kg) performing STST (~10 trials per bird) and used to calculate pelvic limb joint ROMs throughout the movement. Although there was substantial variation in STST motions, some support was found for joint ROMs increasing with body size. As a consequence, muscle-tendon units must generate relatively greater work during the STST in larger birds. However, more biomechanical data from a broader sample of taxa and sizes are needed to conclusively test our hypothesis. If supported, this would imply that the structure of the musculoskeletal system must scale in a way that resolves a compromise of constraints between the demands of economical, fast locomotion (with reduced joint ROMs

in larger species) and non-locomotor motions such as STST (with greater joint ROMs in larger species).

### A8.41 ATOMIC FORCE MICROSCOPE STUDIES OF TREE FROG TOE PADS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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The AFM is a versatile device that provides information on morphology, physical properties and mode of function. This poster features details of the micro- and nanoscale structure of tree frog toe pads, estimates the effective elastic modulus from indentation experiments, and, using special probes with defined tip radii, characterizes the adhesive and frictional properties of toe pad epithelial cells. It brings together work carried out in Aachen with Ingo Scholz, in Kiel with Heinrich Peisker, as well as joint work in Glasgow. Since AFM studies do not require one to work on fixed tissue, fixation artefacts are absent. One can therefore study features of the toe pad epithelial cells surrounded by channels and the morphology of the dense arrays of nanopillars that cover their surface in their natural state. Physical properties of the toe pads are studied by using the AFM as a nano-indenter, the resulting force/distance data allowing one to estimate the stiffness of the pad material. The values for the resulting effective elastic modulus are in the region of 50kPa, making it one of the softest of biological tissues. The use of special probes permits the characterization of toe pad epithelial cell adhesion and friction at the micro- and nanoscale in the fully immersed state. While there is almost complete absence of adhesion under these conditions (capillarity requires an air-water interface), frictional forces are significant. They give rise to friction coefficients of 0.5–1, emphasizing the role of epithelial cell nanostructures for producing this exceptional performance.

### A8.42 A ROBOTIC FOOT DRIVEN BY MUSCULOSKELETAL SIMULATIONS OF FROG JUMPING & TOWARDS MORE REALISTIC ENVIRONMENTAL INTERACTIONS IN FORWARD MODELLING

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Forward dynamic simulation is a powerful tool for predicting joint trajectories under hypothetical physiological conditions. Contrary to experimentation, simulation parameters such as muscle activation and segment lengths can be varied easily to address questions on evolution or performance. However, these simulations often lack realistic substrate interactions. Ground reaction force (GRF) is the fundamental driver of terrestrial locomotion, yet most simulations use simplified contact models that neglect the rich

interplay between limb and substrate. A new method is proposed to generate physical GRF from real substrates, using a robotic frog foot operating in parallel with a real-time simulation: the robotic segment is driven by simulated joint moments, while the resulting robotic deflection updates the motion of the virtual body to simulate jumping. A preliminary system with a single robotic joint and a simplified simulation model was developed to demonstrate the feasibility of hardware driven by virtual dynamics. The current prototype works toward the long-term goal to build a three joint robot capable of translating across a substrate according to a simulated trajectory. The method will determine how various substrates influence GRF characteristics, internal dynamics and emergent limb kinematics to provide new insight into stability and control of frog jumps on compliant substrates. Moreover, this project steps towards a larger ambition of interfacing simulations with real-world inputs and outputs to realistically and representatively bring hypothetical biomechanics to life.

### A8.43 QUANTITATIVE ANALYSIS OF LOCOMOTION AS AN INDICATOR OF BIRD PERSONALITY

WEDNESDAY 6 JULY, 2016 POSTER SESSION

JADE HALL (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), SIOBHAN ABEYESINGHE (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), MONICA DALEY (ROYAL VETERINARY COLLEGE, UNITED KINGDOM)

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Objectively quantifying animal welfare remains a challenge, because welfare assessment requires inferring the subjective experience of animals based on external expression and physiological signs. This problem is especially challenging for animals that are less externally expressive than others, such as avian species with fixed facial features. We aim to develop quantitative indicators of animal welfare based on analysis of locomotor dynamics. Previous literature suggests individuals within species have a predisposition to respond consistently across different contexts (personality). Humans use facial expression to decipher emotion, but also infer personality and emotion from locomotor dynamics. As many aspects of locomotion and physiology are conserved across vertebrates, we propose to develop quantitative indicators of animal emotion and personality based on locomotor dynamics. Here we measure individual variation in expression of bold versus shy personality in guinea fowl (*Numida meleagris*), using both locomotor dynamics and established behavioural indicators. The emergence test and novel environment test were used to evaluate bold-shy personality. We used principle component (PC) analysis to evaluate the correlation among locomotor and behavioural indicators, which revealed that PC1 explained over 50% of the variance among individuals, with high loadings for both behavioural and locomotor measures. This suggests strong correlation among different measurements in discerning bold-shy expression. This preliminary study supports our hypothesis that locomotor dynamics may serve as a useful quantitative metric of bird expression. By continuing to develop locomotion-based indicators for bird personality and emotion, we hope to improve the assessment tools available for welfare monitoring in poultry species.

### A8.44 DISTRIBUTION OF SOUND PRESSURE LEVELS AROUND A SINGING CRICKET: BILATERAL ASYMMETRY IN THE RADIATED SOUND FIELD

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Male field crickets generate mating calls through tegminal stridulation: the rubbing together of the overlying right wing, which bears a file of teeth, against the underlying left wing which carries a scraper. Transmission effectiveness will be maximised by omnidirectional propagation at increased intensities, as the location of the females is unknown to the singing males. However, producing an omnidirectional sound field of maximum sound pressure may be impractical due to the functional asymmetry present in the cricket sound generation system. Functional asymmetry occurs by the right wing coming to partially cover the left wing during the closing stroke phase of stridulation. Therefore it is hypothesised that the sound field on the left-wing side of the animal will contain lower sound pressure components than the right-wing side as a result of this coverage. This hypothesis was tested using an innovative method to accurately record a high resolution, three dimensional mapping of sound pressure levels around field crickets singing under pharmacological stimulation. For acoustic recordings, the robotic arm moved a microphone across a series of positions around a central point, maintaining a consistent distance and aspect to the centre. The results indicate that between individuals, a bilateral asymmetry is present, with higher amplitude components occurring on the right-wing side of the animal, the uncovered wing. Individual variation in the directionality of sound pressure to either the right or left-wing side is also observed. However, statistically significant differences in lateral sound field asymmetry as presented here may not constitute a biologically relevant finding.

## A8.45 TREE FROG ADHESION: THE ROLE OF SUB-ARTICULAR TUBERCLES

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Many climbing animals have more than one type of adhesive organ—e.g. many insects have claws as well as adhesive pads. Their roles are complementary, the former being more effective on rough surfaces, the latter on smooth surfaces. However, the structures of the toe pads and subarticular tubercles of tree frogs are rather similar, though the latter appear rather less specialised (narrower channels between the tubercle epithelial cells and lower densities of the nanopillars that cover the epithelial cell surface). Since the tubercles are better developed in larger species and also increase in size as the frog grows, could it be that their main function is to maintain adhesive ability? We have also found that tubercles come into play when the frog is climbing narrow objects (in our experiments Perspex cylinders). Here there is clear evidence for gripping forces, suggesting that the tubercles might be specialised for friction rather than adhesion. Current work is addressing the extent to which tubercles are used to maintain a grip as the frog (*Litoria caerulea*) is tilted from the horizontal towards the inverted position, as well as their use during climbing Perspex cylinders of varying diameter. Pad and tubercle contact area are measured by the technique of frustrated total internal reflection. Combining such observations with an SEM study of the differences/similarities of pads and tubercles, and measurements of the adhesive and friction forces that they can develop with a miniature (custom-built) force transducer will help to answer the above questions concerning their function(s).

## A8.46 LOSING THEIR LIFELINE? THE EFFECTS OF OCEAN WARMING AND ACIDIFICATION ON MUSSEL ATTACHMENT

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Bivalve mussels often dominate and structure wave swept mid-intertidal zones on temperate coasts and are an important aquaculture species, sustaining a worldwide industry worth over €1.3 billion annually. The secret to the mussels' success is their ability to anchor themselves to rocks with collagen-like fibers (byssal threads) which dynamically absorb wave energy, extending to up to twice their length. Each thread is molded in a pedal groove and tipped with a biological adhesive, made up of proteins which have the unique

ability to adhere to a variety of conventionally challenging surfaces (e.g. glass, plastics, wood, and Teflon), all while in the presence of excess water, salts, and polar organic molecules. Work from our laboratory has shown that the environmental conditions under which they are produced has a profound, region-specific effect on their function. Mussels placed in seawater with high  $p\text{CO}_2$  (low pH, ocean acidification or OA) produce weaker and less extensible byssal threads, lowering overall attachment by 40%. Manipulations of the pH conditions present during byssal adhesive formation suggest that pH may act as a molecular trigger, initiating protein cross-linking. Ocean warming (OW) displays a species-specific effect on byssal thread quality and quantity, dramatically lowering attachment strength in one species (*Mytilus trossulus*) but increasing in another (*M. galloprovincialis*). Our ecomechanical approach helps establish which environmental conditions promote strong byssal attachment which help inform commercial aquaculture facilities about which seawater variables should be monitored to better identify and adapt to unfavorable growing conditions.

## A8.47 THREE-DIMENSIONAL LOCOMOTION OF THE MICROSWIMMER CAENORHABDITIS ELEGANS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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*Caenorhabditis elegans* is an important model for studying undulatory swimming in low Reynolds number environments. Research has focussed on modelling the kinematics of locomotion in response to systematic changes in the mechanical properties of the surrounding fluid, increasing our understanding of the worm's material properties, active control and fluid dynamics. This research has been limited to investigating the worm's movement over agar surfaces, or through thin slabs of fluids where wall effects dominate and animals are constrained to two-dimensional motion. However, under natural conditions *C. elegans* moves through the complex-structured volumetric environments of rotting vegetable matter. To date, accurate three-dimensional high-resolution movement data of *C. elegans* has been lacking, meaning we are potentially missing significant information about the worm's locomotion and behaviour. The capacity of *C. elegans* to move through three-dimensions, the kinematics of locomotion far from any interfaces, and the range of behaviours exhibited in such environments are therefore open questions. To address these we have designed and built a tri-axial microscope system to image a volume significantly larger than the worm at high spatial and temporal resolution. Worms are placed in a glass cubes containing clear fluids with different viscoelastic properties. Three cameras are positioned to face three adjacent sides. Each camera is illuminated with red backlighting, creating a silhouette of the worm in each view. Computer vision is used to identify the position, orientation and configuration of the worm body in three-dimensions within the volume. Preliminary analyses of the three-dimensional kinematic locomotion of *C. elegans* are presented.

## A8.48 MECHANISMS OF SHEAR-SENSITIVE ADHESION IN CLIMBING ANIMALS: PEELING AND SLIDING-INDUCED INCREASE IN ADHESIVE STRENGTH

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Climbing animals can control adhesive forces during locomotion by using shear forces. However, the detailed mechanisms underlying this ability are still not fully understood. We investigated in stick insects (*Carausius morosus*) how shear forces influence adhesion, by performing pull-offs at different retraction angles or with feedback-controlled, constant shear forces. The pull-off forces matched closely the predictions from peeling theory when shear forces were small, but strongly exceeded them when the pads started to slide at higher shear forces. The dramatic increase of adhesion with sliding can be explained by 'pre-stretching' of the pad cuticle and by the depletion of fluid secretion, resulting in a sharp transition from low to high adhesion at a peel angle of ca. 30°. Our results provide an explanation for the tight coupling of adhesion and friction, which is fundamental to adhesion control across all climbing animals.

## A8.49 HOW DO MALE BEETLES PROPEL A HYPER-ELONGATED PENIS INTO A FEMALE DUCT?

WEDNESDAY 6 JULY, 2016 POSTER SESSION

YOKO MATSUMURA (KEIO UNIVERSITY, JAPAN), JAN MICHELS (KIEL UNIVERSITY, GERMANY), ALEXANDER FILIPPOV (DONETSK INSTITUTE FOR PHYSICS AND ENGINEERING, UKRAINE), ALEXANDER KOVALEV (KIEL UNIVERSITY, GERMANY), THERESA GÖDEL (KIEL UNIVERSITY, GERMANY), ESTHER APPEL (KIEL UNIVERSITY, GERMANY), STANISLAV GORB (KIEL UNIVERSITY, GERMANY)

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The occurrence of males having a hyper-elongated penis with a length of several times their body length is widespread in the animal kingdom, especially in insects. This phenomenon helps males to increase their probability of paternity by being chosen by females. Despite of these advantages, male insects with such a hyper-elongated penis are challenged by several length-related problems, such as the storage of the penis in their abdomen and its insertion into the female genitalia with precise penile propulsion control. To examine how males propel their hyper-elongated penis into female genitalia, we examined the reproductive system of Cassida beetles by using different techniques including microscopy, computer tomography and computer simulations. The results show that muscles surrounding the penis are responsible for generating the propulsion force of the penis. Moreover, a bending test and a material composition analysis using autofluorescences revealed a stiffness gradient along the penis, with the tip being softer than the rest. We performed a numerical simulation imitating the beetle

system and demonstrated that a penis with such a stiffness gradient penetrates female genitalia faster compared with penises featuring other hypothetical stiffness conditions. It is likely that the stiffness gradient helps the penis to adapt to the complicated female genital shapes. In conclusion, the propulsion of the hyper-elongated penis is simply based on the contraction of the muscles surrounding the penis and supported by the stiffness gradient of the penis.

## A8.50 HIGH RESOLUTION THREE-DIMENSIONAL SURFACE MEASUREMENTS OF BIRDS OF PREY IN GLIDING FLIGHT

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Accurately measuring the wing shape of flying animals is of great importance for accurate aerodynamic analysis. This is because the flow phenomena and resulting forces and moments are sensitive to subtle changes in shape. Historically, aerodynamic analysis of birds has mostly been reliant on approximate models of wing geometry or the use of animal cadavers placed in approximate flight configurations. Both of these approaches suffer the limitation that they are unlikely to accurately reproduce the in-flight geometry. Here, a new method for high resolution three-dimensional geometric measurement of free-flying birds is presented. A trained barn owl (*Tyto alba*) and peregrine falcon (*Falco peregrinus*) were flown outdoors past a set of eight synchronised DSLR cameras arranged in pairs above and below the bird's flight path. The surface geometry of the steadily gliding bird (~1 million points) was measured using the new photogrammetric technique which is based on a phase correlation approach. The demonstrated accuracy of this new method is +/-2.5mm for 95% of the points based on measurement of a life size bird model made under field conditions. The accuracy and resolution of the measurements far exceed anything so far achieved in bird flight research, and stands to significantly improve the accuracy of future analysis of bird flight dynamics.

## A8.51 PUSH OR PULL? THE LIGHT-WEIGHT ARCHITECTURE OF THE *DAPHNIA PULEX* CARAPACE IS ADAPTED TO WITHSTAND TENSION, NOT COMPRESSION

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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*Daphnia* (Crustacea, Cladocera) are prominent for their ability to form morphological adaptations thwarting the threat of coevolved predators. In addition to spines and helmets, the carapace, encapsulating the main body, offers protection. It is an evagination of the head integument and thus a double layer of the integument. The two integumental layers are interconnected by small pillars, which were previously described as providing higher mechanical stability against compressive forces. Following this hypothesis, we analyzed the carapace structure using histochemistry in combination with light and electron microscopies. Furthermore, we measured the hemolymphatic gauge pressure, because the space between the integumental layers of the carapace is filled with hemolymph. We found the distal integument of the carapace to be significantly thicker than the proximal. The pillars appear fibrous with slim waists and broad, sometimes branched bases where they meet the integument layers. Our findings brought up a new idea about the functionality behind the carapace stability in *Daphnia*.

## A8.52 A SIMPLE MODEL FOR ENERGETICALLY-OPTIMISED JUMPING INVESTIGATED IN DOGS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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The ability to jump over raised obstacles in the path of travel is a useful modulation of regular gait. The mechanical energy required to jump over an obstacle is minimised by a single trajectory resulting from a unique velocity at take-off. The familiar result that the trajectory is a parabola with its apex coincident with the zenith of the obstacle applies only when take-off position is unconstrained. Using a simple ballistic model we show that the apex of the lowest energy trajectory occurs before the zenith of the obstacle when take-off position cannot be arbitrarily close to the obstacle. We investigated whether domestic dogs (*Canis lupus familiaris*) utilised jump trajectories that minimised mechanical energy at take-off when traversing an obstacle with a constrained take-off region. The kinematics of five dogs were recorded as they traversed a raised obstacle using different take-off positions. Jump trajectories were compared with the predictions of the ballistic optimisation model

using experimentally-determined take-off parameters. We found that the dogs systematically modified their jump apex position relative to the obstacle in the predicted direction in response to changes in obstacle geometry. CoM trajectories were close to those predicted to minimise the mechanical energy cost of the jump for a range of obstacle lengths. It is unclear how the dogs acquired the complex perception and control necessary to exhibit the behaviour observed in this study. The model may be used to investigate the energetic optimisation any similarly-constrained ballistic task.

## A8.53 LONGITUDINAL BALANCE OF THE GREAT HAMMERHEAD (*SPHYRNA MOKARRAN*)

WEDNESDAY 6 JULY, 2016 POSTER SESSION

GIL IOSILEVSKII (TECHNION, ISRAEL)

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The great hammerhead is denser than water and hence relies on hydrodynamic lift to counteract gravity. The lift is generated mainly by the pectoral fins, the cephalofoil, and the heterocercal tail. The lift of the fins and the cephalofoil is determined by their orientation relative to the body and by the angle of the body relative to the direction of swimming; the lift of the tail is adjusted accordingly to retain the longitudinal balance of the shark. In this study, we have placed a morphologically accurate model of the great hammerhead in a wind tunnel, and measured forces and moments acting on the shark for different orientations of the pectoral fins and the cephalofoil relative to the body, and of the body relative to the flow. The Reynolds number in all the experiments was similar to that of a free-swimming shark. The most conspicuous findings can be summarized as follows. (i) At cruise speeds, the lift of the tail is estimated to be less than 25% of the total lift, practically independent of the orientation of the pectoral fins relative to the body. (ii) Raising or lowering the head can increase or decrease the lift of the tail by 5% of the total lift. (iii) Aligning the pectoral fins with the flow when the body is at angle to it does not remove the lift generated by the fins. (iv) The cephalofoil increases drag by 10%, but reduces the minimal swim speed by almost 30%.

## A8.54 CAN SERIES ELASTIC ELEMENTS AMPLIFY MUSCLE POWER IN COLD LIZARDS?

WEDNESDAY 6 JULY, 2016 POSTER SESSION

JORDAN BALABAN (UNIVERSITY OF CALIFORNIA IRVINE, UNITED STATES), EMANUEL AZIZI (UNIVERSITY OF CALIFORNIA IRVINE, UNITED STATES)

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Western fence lizards maintain nearly the same maximal running speed between 25-40°C despite substantially slower muscle contractile speeds at low temperatures. Though muscle kinetics allow stride frequency to be maintained down to 25°C, the power requirements to accelerate and maintain velocity may not be met by muscle alone at low temperatures. We hypothesize that lizards use series elastic elements (SEE) such as tendons and aponeuroses to amplify muscle power and maintain performance at low and

intermediate temperatures. To test this hypothesis, we filmed lizards running on sandy and hard substrates at 15, 25, and 35°C. Since loading of the SEE requires a large ground reaction force, lizards are more likely to amplify power on hard substrates compared to sand. Therefore, if lizards are using elastic elements to amplify power, we predict significant differences in running speed and acceleration between hard and sandy substrates at 15°C and 25°C. At higher temperatures performance differences may be minimal as muscle power is less limiting to speed and acceleration. Preliminary results support our hypothesis as running speed at 15°C and 25°C is higher on hard substrates compared to sand. We see no difference in maximal running speed between either substrate at 35°C. There also appears to be much higher peak accelerations on hard surfaces at all temperatures, with similar accelerations at 25°C and 35°C. These results provide preliminary support that elastic energy storage may be used to maintain performance across a broader thermal range.

### A8.55 SKELETAL MUSCLE ARCHITECTURE DETERMINES PROPENSITY FOR MUSCLE DAMAGE DURING ECCENTRIC CONTRACTIONS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

EMILY M ABBOTT (UNIVERSITY OF CALIFORNIA IRVINE, UNITED STATES), ITOHAN AIKHIONBARE (UNIVERSITY OF OREGON, UNITED STATES), MANNY AZIZI (UNIVERSITY OF CALIFORNIA IRVINE, UNITED STATES)

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Skeletal muscles, paired with springy tendons, decelerate our bodies during movement. During decelerating tasks, such as landing and braking, muscles stretch while producing force to dissipate mechanical energy. This active lengthening can cause muscle damage. While we know how different architectures and fibre-type compositions affect force production, it is unclear how these muscle properties affect the likelihood of damage. Is there a link between muscle properties and injury? We used the natural variation in muscle fibre-type and architecture in rat muscle tendon units (MTUs). Soleus (SOL) is a slow, parallel fibre muscle (20% fast fibres, 4° pennation) while plantaris (PL) is a fast, pennate fibre muscle (95% fast fibres, 16° pennation). We used an in situ muscle preparation where a servomotor measured the force, velocity and length of the entire MTU. Muscles were actively stretched until they reached 130%, 150% or 170%  $P_0$ . After an eccentric contraction, the extent of the muscle injury was estimated by the amount of stress decline. For example, after a 170%  $P_0$  eccentric contraction, PL lost  $2.21 \pm 0.19$  N/cm<sup>2</sup> stress (10.75%  $\pm$  1.13%) and SOL lost  $10.47 \pm 1.04$  N/cm<sup>2</sup> stress (53.27%  $\pm$  4.03%). These results suggest that variation in muscle architecture may serve to predict a muscle's propensity for damage during decelerating tasks. This is consistent with studies that show an increase in pennation angle with eccentric training.

### A8.56 THE AVERAGE AREA OF INDIVIDUAL SECONDARY OSTEONS SCALES ALLOMETRICALLY IN MAMMALIAN LIMB BONES

WEDNESDAY 6 JULY, 2016 POSTER SESSION

ALESSANDRO FELDER (THE ROYAL VETERINARY COLLEGE, UNITED KINGDOM), JOHN R HUTCHINSON (THE ROYAL VETERINARY COLLEGE, UNITED KINGDOM), MICHAEL DOUBE (THE ROYAL VETERINARY COLLEGE, UNITED KINGDOM)

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Whole bone shape and micro-structural properties are key determinants of bone strength. Bone shape has been studied extensively in a comparative context. Inter-species scaling relationships of cortical bone micro-structure are less well understood. The creation of secondary osteons during intra-cortical bone remodelling, when packets of new bone tissue replace older bone tissue, is thought to be crucial to maintain bone strength despite frequent repetitive cyclic loads. We investigated the relationship between body mass ( $M$ ) and two micro-structural parameters associated with bone remodelling (average area of one intact secondary osteon (On. Ar.) and percent osteonal infilling) in mammalian limb bones. Using histomorphometric data from novel microscopy images of historical thin sections from 43 mammalian species retrieved from the Quekett Collection of the Royal College of Surgeons of England, we performed a scaling analysis, finding that osteon area scales with negative allometry (On. Ar.  $\propto M^{0.24}$ ,  $R^2 = 0.51$ ,  $p < 0.001$ ), i.e. becoming relatively smaller as body size increases, while percent osteonal infilling is independent of species size. Together, these data suggest that the secondary osteons of larger species have a larger (in absolute terms) distance between the central (Haversian) canal and the most distant osteocytes, but maintain a similar (relative) porosity to small species. We discuss the potential implications this may have on the mechanical strength and the blood perfusion of bone in various mammals.

## A8.57 ADAPTIVE FLIGHT BEHAVIOUR OF URBAN GULLS USING OROGRAPHIC LIFT

WEDNESDAY 6 JULY, 2016 POSTER SESSION

👤 CARA J WILLIAMSON (UNIVERSITY OF BRISTOL, UNITED KINGDOM), EMILY LC SHEPARD (UNIVERSITY OF SWANSEA, UNITED KINGDOM), SHANE P WINDSOR (UNIVERSITY OF BRISTOL, UNITED KINGDOM)

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Birds are known to adapt their flight strategies at regional and global levels to reduce travel costs however little is known about local daily movement. It would follow that gulls modulate their behaviour at the fine scale but due to the stochastic nature of wind this area is relatively unknown. This research investigates the 3D trajectories of gulls commuting along an urbanised seafront in a wide range of wind conditions. We found that gulls systematically adapt their flight paths to utilise the orographic lift generated by a row of seafront hotels. A fine scale wind model generated with simplified computational fluid dynamics verified that gulls reduce their energy costs during regular local flights, making use of the available updraft to maintain altitude at equilibrium glide. Not only do the gulls change their flight paths to make use of the available wind energy but we also see adaptive flight strategies within this. The gulls vary their position within the available updraft to maintain a favoured airspeed and to improve robustness against meteorological variability. Holding position high above the hotels results in a self-regulating phenomenon, in which the gulls are able to maintain equilibrium glide when subjected to horizontal or vertical gusting. Understanding gull flight strategies could prove invaluable for Unmanned Air Vehicles where range and endurance is limited by battery technology. Path planning algorithms for UAVs based on the adaptive behaviour of gulls could improve flight performance by conserving energy and robustness to gusting.

## A8.58 ARE PROFILES OF DRAGONFLY WINGS APPLICABLE TO BIRDS OR AIRPLANES?

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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In comparison to common airfoil profiles of birds and planes with blunt leading edge, smooth surface and sharp trailing edge, profiles of dragonfly wings show a corrugated structure with sharp edges. A number of publications deal with the aerodynamics of these profiles. This contribution intends to answer the question, whether dragonfly profiles are applicable to birds or airplanes.

Using computational fluid dynamics flow fields as well as drag coefficients are calculated for a dragonfly profile of 10% thickness and a corresponding NACA0010 profile for gliding flight and zero incidence. The investigations are carried out for Reynolds numbers ( $Re$ ) of 200 to 20000. Dragonflies range between 100 and 10000. In general, birds and airplanes operate at higher values.

For  $Re=200$  results reveal slight differences in drag coefficient for the investigated profiles. In both cases the thickness of the viscous layer is in the order of the thickness of the profiles. The corrugated structure is fully embedded in this layer, and weak recirculation domains occur in the cavities. With rising  $Re$ , NACA0010 shows a stronger decrease in drag coefficient. The growing difference results from the diminishing thickness of the viscous layer. This leads to increased flow separation in the cavities and higher momentum transport to the profile in comparison to NACA0010. The results indicate, that a corrugated profile is suitable for lower  $Re$ . Common profiles may show lesser drag at higher  $Re$ , which leads to lower energy demand for transport.

## A8.59 FLIGHT POWER MUSCLES IN THE DIPTERA: ARE THEY OPTIMALLY ORIENTED FOR A RESONANT SYSTEM?

WEDNESDAY 6 JULY, 2016 POSTER SESSION

ANNA CHABOKDAST (UNIVERSITY OF OXFORD, UNITED KINGDOM), SIMON WALKER (UNIVERSITY OF OXFORD, UNITED KINGDOM)

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The dipteran flight motor is regularly described as operating as a resonant system. The necessary opposing driving forces are provided by two groups of stretch-activated power muscles, the dorsal longitudinal muscles (DLMs), and the dorsoventral muscles (DVMs). The relative size and direction of these muscles represents their force production capability, which is important in determining the function and efficiency of a resonant system. We used micro-CT scans of blowflies to virtually segment and measured the power muscles and found that although the volume of the two groups of muscles are comparable, DLMs are slightly larger than DVMs (54% vs 46%). Furthermore, despite typically being described as orthogonal, the DLMs and DVMs are only angled c. 45° to each other. To investigate the effect of DVMs alignment on the function of the resonant system, we developed a simplified Multibody Dynamic model of the thorax consisting of a four-bar linkage system and two sets of muscles. We found that a model with the actual orientation of the DVMs resulted in significantly smaller excitation of both muscles and ultimately smaller thorax deformation compared to a model with orthogonal orientation. We therefore suggest that the configuration of the power muscles is not optimized purely for the purpose of maximum thorax deformations in the resonant system, although the influence of other forces, e.g. due to elastic storage, remains to be known.

## A8.60 MOTION AND DEFORMATION OF THE DERMAPTERAN HIND-WING

WEDNESDAY 6 JULY, 2016 POSTER SESSION

JULIA DEITERS (UNIVERSITY OF DUISBURG-ESSEN, GERMANY), TOBIAS SEIDL (WESTPHALIAN UNIVERSITY OF APPLIED SCIENCE, GERMANY), WOJCIECH KOWALCZYK (UNIVERSITY OF DUISBURG-ESSEN, GERMANY)

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In the insect's wing, musculature can only be found at the wing base. When the insect flies, the entire wing needs to adapt passively to the aerial forces emerging from the flapping movements. This results in considerable shape changes and a movement delay of the wing tip in relation to the wing base. In addition, structures which facilitate folding and unfolding wings influence the three-dimensional shape of the wing and the ability to deform the wing.

Earwigs which have highly foldable hind-wings use two different mechanisms to lock the wing in its deployed state. Unlocking and folding is usually initiated through an attachment impact at one of these locking mechanisms and by resilin filled veins. Little is known about their general aerial performance. Therefore, we conducted three-dimensional high-speed cinematographic studies on *Labia minor*. Wing movements were tracked by marking points of the trailing edge. Through their displacement, wingspeed, wingbeat frequency, wing tip path, and the angle of the wing in relation to the body can be determined.

We found that earwigs' wings are significantly deformed in their flight similar to many so called 'slow flying' insects. A 'snap-out' of the locking mechanisms could be easily induced through structural deformation. Therefore, it is a great structural challenge for the earwigs' wings to remain unfolded and not to collapse. In order to determine the magnitude of the deformation, a CAD-Modell in NX was generated, and a multi-body simulation with flexible elements was performed.

# A9 OPEN ANIMAL BIOLOGY

**ORGANISED BY:** DR PETER HUBBARD (UNIVERSITY OF ALGARVE, PORTUGAL)  
AND DR LYNNE SNEDDON (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM)

## A9.1 LARGE MEALS INCREASE DIGESTION EFFICIENCY BUT OCCUPY THE MAJORITY OF AEROBIC SCOPE IN A TROPICAL PREDATORY FISH

📅 WEDNESDAY 6 JULY, 2016 ⌚ 9:00

👤 TOMMY NORIN (UNIVERSITY OF GLASGOW, UNITED KINGDOM),  
TIMOTHY D. CLARK (UNIVERSITY OF TASMANIA AND CSIRO  
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Feeding is an essential part of life for all animals as it provides energy for activity, growth and reproduction. However, digestion itself elicits an energetically costly metabolic response, the 'specific dynamic action' (SDA), which at its peak may take up a substantial proportion of an animal's overall capacity for oxygen transport (its aerobic scope) and compromise other activities. By measuring the SDA response of 24 juvenile barramundi (*Lates calcarifer*) fed different sized meals ranging from 0.6 to 3.4% of body mass (percentage of dry feed to fish wet weight), we found that individuals used more energy overall to digest larger meals (SDA vs. meal size;  $r^2=0.895$ ,  $P<0.0001$ ), but the percentage of digestible meal energy used in the SDA process decreased asymptotically with increasing meal size (SDA coefficient vs. meal size,  $r^2=0.765$ ,  $P<0.0001$ ). Growth also increased with meal size ( $r^2=0.624$ ,  $P<0.001$ ). These results suggest that it is energetically advantageous for barramundi to select large prey. However, during the peak of SDA following a large meal, digestion occupied as much as 77% of the available aerobic scope (compared to ~30% in fish digesting small meals). This suggests that other aerobic activities will be compromised after ingestion of a large meal and instead points to a disadvantage of selecting large prey. The existence of this metabolic trade-off between meal size and other important activities like swimming and predator evasion suggests that barramundi would benefit from regulating their prey size as a function of imminent requirements and threats in their environment.

## A9.2 RED BLOOD CELLS OPEN PROMISING AVENUES FOR LONGITUDINAL STUDIES OF AGEING IN CAPTIVE AND WILD VERTEBRATES

📅 WEDNESDAY 6 JULY, 2016 ⌚ 9:15

👤 ANTOINE STIER (INSTITUTE OF BIODIVERSITY ANIMAL  
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GLASGOW, UNITED KINGDOM), SOPHIE REICHERT (DEPARTMENT  
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UNITED KINGDOM), FRANÇOIS CRISCUOLO (DÉPARTEMENT  
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Ageing is characterized by a progressive deterioration of multiple physiological and molecular pathways, which impair organismal performance and increase risks of death with advancing age. Hence, ageing studies must identify physiological and molecular pathways that show signs of age-related deterioration, and test their association with the risk of death and longevity. This approach necessitates longitudinal sampling/monitoring of the same individuals with advancing age. Indeed, the selective disappearance with time of particular individuals is likely to bias the results about age-related variations of physiological markers coming from cross-sectional studies (i.e. comparing individuals for different age groups). Moreover, investigating relationships between physiological markers and survival requires the monitoring of individuals on the long-term. Such longitudinal sampling however requires a minimally invasive sampling technique that provides access to the larger spectrum of physiological processes and molecular pathways being putatively associated with ageing. This talk will underline the interest in using red blood cells (RBCs) as a promising target for longitudinal studies of ageing in vertebrates. RBCs could provide valuable information on the following pathways: cell maintenance and turnover (RBC number, size, heterogeneity and renewal rate); glucose homeostasis (RBC glycated haemoglobin); oxidative stress parameters (antioxidant defences and oxidative damage); cellular stress resistance; mitochondrial functioning, and telomere dynamics. The last two pathways are specific to RBCs of non-mammalian species, which possess a nucleus and functional mitochondria. I will present an overview of the current knowledge about RBCs and age-dependent changes in the aforementioned pathways, but also on how they could relate to survival and lifespan.

### A9.3 BEETLE! – CLOSE YOUR SPIRACLES AND PROTECT YOUR TRACHEAL SYSTEM AGAINST PARASITE INFESTATION

WEDNESDAY 6 JULY, 2016 9:30

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In insects, gas exchange between atmosphere and tissues occurs via a tracheal system. The spiracles, gate like structures, are located on the thoracic and abdominal part of insects' body. Proportion of times spiracles become open defines the three possible gas exchange patterns that insects employ at rest: discontinuous (DGE), cyclic and continuous. DGE is characterized by periodic sustained spiracle closure with zero organism-to-environment gas exchange. Although DGE is widely documented, its physiological and evolutionary costs and benefits remain unresolved. We provide support for a previously untested hypothesis that posits that DGE minimizes the risk of infestation of the tracheal system by mites. Here, we analyze the respiratory patterns of 15 species of ground beetle (Carabidae), of which more than 40% of individuals harbored external mites. Beetles employed DGE significantly more often when harboring external mites in comparison with individuals not carrying mites ( $p < 0.001$ ). Mite-free individuals predominantly employed a cyclic or continuous gas exchange pattern, which did not include complete spiracle closure. The ability to show DGE with sustained periods of spiracle closure may reduce invading, clogging or transferring pathogens to the tracheal system, which can undoubtedly reduce host evolutionary fitness.

### A9.4 BLOOD FLOW AND THE DEVELOPMENT OF ANEURYSMS: NOVEL INVESTIGATIVE MEASUREMENTS

WEDNESDAY 6 JULY, 2016 09:45

HANNAH SAFI (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), NATHAN PHILLIPS (ROYAL VETERINARY COLLEGE, UNITED KINGDOM), RICHARD J. BOMPHREY (ROYAL VETERINARY COLLEGE, UNITED KINGDOM)

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An Abdominal Aortic Aneurysm (AAA) occurs when the wall of the artery dilates into a balloon-like bulge. An AAA is defined as an enlargement of the aorta by at least 1.5 times its original diameter in the maximum transverse dimension. In the clinical setting clinicians must decide when the risk of an AAA rupture justifies the risks associated with surgical repair. Yet, at present there is no accepted method to quantify the risk of rupture for individual

AAAs. Elective repair decisions are generally founded on the 'maximum diameter criterion', typically 5.5 cm. However, this criterion is a general rule-of-thumb and known to be unreliable because AAAs smaller than this threshold diameter can also rupture. A biomechanics-based approach to rupture prediction built on computational models can be applicable in the clinical setting. Recent guidelines for treatment of AAAs from the Society for Vascular Surgery suggest computationally acquired rupture predictors need further validation prior to their implementation in a clinical setting. Here, we present an emerging technique where simultaneous fluid flow and atrial wall strain measurements are carried out using the methods of Particle Image Velocimetry and Digital Image Correlation respectively. Measurements are tested on a highly simplified silicone AAA model. We demonstrate this combined technique for investigating the fluid-structure interactions between blood flow and vessel wall deformation. Visualising the experimental physical modelling of internal flows and wall surface deformations highlights great potential in validating computational models.

### A9.5 ELEVATED BLOOD VISCOSITY CAUSES CARDIOVASCULAR COLLAPSE IN EMBRYONIC CHICKENS

WEDNESDAY 6 JULY, 2016 10:00

ZACHARY A KOHL (UNIVERSITY OF NORTH TEXAS, UNITED STATES), DANE A CROSSLEY II (UNIVERSITY OF NORTH TEXAS, UNITED STATES)

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Blood viscosity, a key component of vascular resistance, increases from 1.6 mPa·s in embryonic chickens to 3.7 mPa·s in adult chickens. This increase in viscosity is not attributed to hematocrit given that over the same time period blood viscosity increases 230% while hematocrit increases from 32 to 37%. Therefore, embryonic chickens maintain a similar hematocrit to adults but with a largely reduced cost of transport. We sought to understand the consequences of embryonic hyperviscosity through acutely induced changes in blood viscosity by the infusion of isosmotic 6% Dextran (3 ml/kg embryo wet mass in 0.6% saline) into a chorioallantoic membrane artery in 90% developed chickens. Dextran is a complex polysaccharide clinically used as an emergency volume expander. We hypothesized that embryonic chicken cardiovascular systems would be unable to cope with 'adult' values of blood viscosity resulting in acute cardiovascular collapse. Our data indicated hyperviscosity caused a shift from intermittent to tonic vagal function, demonstrated by an approximately 15% increase in minute heart rate following cholinergic receptor blockade. Additionally, we identified that Dextran can be used as a non-pharmacological method of assessing baroreflex function. Overall, our results indicate that chicken embryos are unable to maintain sustained cardiovascular function following a 30% increase in blood viscosity.

## A9.6 REGULATION OF GENE EXPRESSION IN FISH RED BLOOD CELLS

WEDNESDAY 6 JULY, 2016 10:15

MIRIAM GÖTTING (UNIVERSITY OF TURKU, FINLAND)

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Gene expression is traditionally divided into several stages, including mRNA synthesis and processing, translation, and decay. All of these stages are coupled and are tightly regulated. The rate of mRNA synthesis and decay determine the steady-state level of mRNA.

In fish red blood cells (*Oncorhynchus mykiss*) we investigated the different stages of regulation in response to various environmental signals, such as normoxic and hypoxic conditions as well as under  $\beta$ -adrenergic stimulation. While there are changes in the transcription rate due to treatment, the steady state levels are barely affected. Determined mRNA decay rates (transcript half-life) resemble very well the function of the gene products. Transcripts of Hif1a and Na<sup>+</sup>/H<sup>+</sup> exchanger are stabilized under hypoxia and  $\beta$ -adrenergic stimulation, while the  $\beta$ -adrenergic receptor is only stabilized under adrenergic stimulation. In Hif1a we furthermore studied the effects of temperature on the various stages of gene expression. Our data suggest that the steady-state mRNA levels cannot serve as a reliable assay to examine transcription or decay rates. Red blood cell transcript levels seem to be robust to changes in either transcription rate or decay or both.

## A9.7 *IN SITU* CARDIAC PERFUSION REVEALS INTERSPECIFIC VARIATION OF INTRAVENTRICULAR FLOW SEPARATION IN REPTILES

WEDNESDAY 6 JULY, 2016 11:00

WILLIAM JOYCE (AARHUS UNIVERSITY, DENMARK), MICHAEL AXELSSON (UNIVERSITY OF GOTHENBURG, SWEDEN), JORDI ALTIMIRAS (LINKÖPING UNIVERSITY, SWEDEN), TOBIAS WANG (AARHUS UNIVERSITY, DENMARK)

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The hearts of non-crocodylian reptiles are comprised of two atria and an incompletely divided ventricle, and thus present an ideal paradigm to explore the evolution of the double circulation in vertebrates. In this study, we used an *in situ* double-perfused heart preparation to evaluate intraventricular flow separation in five reptiles species (turtle: *Trachemys scripta*, rock python: *Python sebae*, yellow anaconda: *Eunectes notaeus*, varanid lizard: *Varanus exanthematicus*, and bearded dragon: *Pogona vitticeps*). To simulate changes in vascular bed resistance, pulmonary and systemic afterloads were independently manipulated and changes in blood flow distribution amongst the central outflow tracts were monitored. Rock pythons and varanid lizards exhibited pronounced intraventricular flow separation. As pulmonary or systemic afterload was raised, flow in the respective circulation decreased. However, flow in the other circulation, where afterload was constant, remained stable. This correlates with the convergent evolution of intraventricular pressure separation and the large intraventricular muscular ridge, which compartmentalises the ventricle, in these

species. Conversely, in the three other species, the pulmonary and systemic flows were mutually dependent, such that the decrease in pulmonary flow in response to elevated pulmonary afterload resulted in redistribution of perfusate to the systemic circuit (and vice versa). Thus, in these species blood can readily transverse the intraventricular cava. Our study emphasises that the independent evolution of functionally similar intracardiac flow separation in lizards (varanids) and snakes (pythons) from an ancestor endowed with a large capacity for intracardiac shunts only required relatively minor structural modification in cardiac structure.

## A9.8 TEMPERATURE-DEPENDENT MORPHOLOGICAL REMODELLING OF FISH CARDIAC MITOCHONDRIA

WEDNESDAY 6 JULY, 2016 11:15

ALEXANDER J HOLSGROVE (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), GINA LJ GALLI (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), HOLLY A SHIELS (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

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Cardiac remodelling in response to thermal acclimation has been displayed in a number of fish species, with many presenting hypertrophy in response to chronic cold and the opposite in response to chronic warming. As cardiac remodelling events alter energetic requirements, the plasticity of metabolic processes may underpin the cardiac phenotype. Despite their pivotal role as the major suppliers of cellular energy, comparatively few studies have investigated the effects of temperature acclimation on cardiac mitochondria in fish. From mammalian studies we understand that mitochondrial morphology is fundamentally linked to mitochondrial function and yet little is known about these processes in ectotherms. The physiological 'status' of cold induced cardiac hypertrophy is still debated, with studies presenting both pathological and physiological traits. The investigation of mitochondrial morphology may help to determine the physiological 'status' of the thermally acclimated fish heart. Rainbow trout *Oncorhynchus mykiss* were acclimated to cold (5°), control (10°) and warm (18°) temperatures to induce cardiac remodelling. Hearts were fixed and sectioned for TEM imaging. Parameters measured including mitochondrial size ( $\mu\text{m}$ ), number and cristae density were measured for each treatment group. The results suggest that cold induced hypertrophy increases mitochondrial number, but has no effect on size. Micrograph results are supported by protein expression data. Together the data indicate that mitochondria increase in number to fuel the energetic cost of cardiac hypertrophy in the cold.

## A9.9 ON BEING THE RIGHT SIZE: CONSEQUENCES OF BODY SIZE AND TEMPERATURE FOR THE ENERGY METABOLISM OF AQUATIC ECTOTHERMS

WEDNESDAY 6 JULY, 2016 11:30

WILCO CEP VERBERK (RADOUD UNIVERSITY NIJMEGEN, NETHERLANDS), DAVID ATKINSON (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM)

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Oxygen is essential for burning food and generating energy, but may become limiting for organisms relying on gas exchange under water. This is because breathing under water is challenging: the diffusion of oxygen is orders of magnitude lower in water than in air, while the higher density and viscosity of water greatly enhance the cost of breathing. However, while a shortage of oxygen quickly leads to asphyxiation, too much oxygen is toxic. Therefore, the ability to regulate oxygen consumption rates is at a premium, enabling ectotherms to balance oxygen toxicity against the risk of asphyxiation across a wide range of temperatures. Although effects of body size and environmental temperature on energy metabolism are well recognised in ectotherms, the situation is more complicated in water. Temperature affects the availability of oxygen in water and the cost of breathing by changing the viscosity. The consequences of such changes are dependent on body size. As a result, a larger body size may represent a respiratory advantage that helps aquatic ectotherms to overcome the larger viscous forces in cold water. This mechanism may help explain why size clines along temperature and latitudinal gradients are much more pronounced in aquatic ectotherms, why gigantism is especially prevalent in aquatic ectotherms, and why mass-scaling exponents often change with temperature. As body size is a major driver of how ecosystems function, understanding how body size is tied to energy budgets in aquatic and terrestrial ectotherms will greatly increase our ability to predict the consequences of global warming.

## A9.10 BITE ME: DOMESTICATION EFFECT ON BITING PERFORMANCE AND AGGRESSION IN RATS

WEDNESDAY 6 JULY, 2016 13:50

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By domestication of wild animals, humans have improved suitable food resources, modes of transportation, companionship and/or group defence against potential threats. Thereby, a wide variety of breeding lines were differentiated by morphological, physiological, genetic and behavioural features. Here, the *in vivo* incisor biting

performance and head morphology of male and female rats (*Rattus norvegicus*) were investigated. Samples were derived from two wild type breeding lines selectively bred for aggressive and tame behaviour towards humans. In addition to this there was a laboratory bred intercross line. While the aggressive and intercross lines showed levels of aggressiveness, none of the tame rats bit. Within intercross and aggressive lines, females produced stronger and more frequent bites than males, whilst aggressive females did so more than intercross females. No intercross-aggressive difference was found in males. In contrast, body size, head length and mandibular width showed clear sexual dimorphism (males > females). The only clear inter-line morphological difference was body size, being the tame rats also the largest. Finally, bite force was not predicted well by any of the head measurements. Thus, beyond genetics and physiology, the long-term domestication process resulted mainly in behavioural differences. It is likely that the larger weight of the tame rats is due to lack of activity. Overall, our results on biting performance cannot be attributed to anatomical differences. Our findings offer additional evidence of dimorphic aggressive behaviour in female rats associated with maternal care and social structure as has been observed in gregarious wild rodents.

## A9.11 MARINE BIRDS SLEEPING AT SEA

WEDNESDAY 6 JULY, 2016 14:05

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King penguins spend weeks at sea, travelling hundreds of kilometers in search of aggregates of fish and squid, and then perform hundreds of dives each day. After such intensive days the penguins rest at the water's surface during the night, diving only rarely. During the night they are likely to spend some time sleeping. While asleep, king penguins intermittently rest with their head in the water and exhale. This lasts for around 15 seconds. They then raise their heads, inhale, and again lower their heads into the water. Because the penguins must remain alert to raise their heads to breathe, we believe that they exhibit uni-hemispheric sleep. Our study focused on the resting behaviour of the king penguins in a sea water tank (at 4°C) and within an enclosure on land, to establish whether they sleep on water and if this is less deep than their sleep on land, measured by arousal threshold. We equipped 20 penguins with accelerometers, body temperature and heart rate loggers. Furthermore, we filmed them continuously to correlate accelerometry data with their behaviour and body posture in the water. We played sounds to establish the arousal threshold during their time in our sea water tank, and in our enclosure on land. Penguins resting on water have an increased arousal threshold. Furthermore, they respond more often and quicker to lower frequencies than when they rest on land.

## A9.12 FEEDING BEHAVIOUR AS AN INDICATOR OF PAIN PERCEPTION IN THE BALL PYTHON (*PYTHON REGIUS*)

WEDNESDAY 6 JULY, 2016 14:20

LAUREN E JAMES (AARHUS UNIVERSITY, DENMARK), CATHERINE JA WILLIAMS (AARHUS UNIVERSITY, DENMARK), MADS F BERTELSEN (COPENHAGEN ZOO, DENMARK), TOBIAS WANG (AARHUS UNIVERSITY, DENMARK)

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The use of reptiles in comparative physiology is well established, however it remains challenging to assess whether a reptile is in pain as a result of recognised experimental protocols. Using withdrawal reflexes and physiological responses to indicate pain perception and to test the efficacy of analgesic agents has thus far led to inconclusive results, particularly in snakes. To refine experimental procedures on reptiles, objective methods of pain monitoring and management are key. Here, we present the potential use of routine feeding behaviour as an adjunct to current pain assessment protocols. Feeding is easily and habitually monitored in both clinical and research environments, thus providing an optimal behaviour to investigate. The aim of this study was to examine whether chemical (capsaicin injection) or physical (surgical incision) noxious stimulation would elicit a delay in feeding behaviour in the ball python (*Python regius*), a snake frequently used in physiological research. The administration of anaesthesia alone had minimal effect on feeding, whereas normal feeding did not resume until 1 and 3 weeks later following a chemical (remote capsaicin injection) or a surgical (sham catheter placement surgery) stimulus, respectively. The surgical stimulus significantly affected feeding behaviour ( $p=0.01$ ), and when a different group of animals was subjected to the same stimulus, with local anaesthesia (bupivacaine 2mg/kg), this alteration to feeding behaviour was significantly reduced ( $p=0.006$ ). These findings demonstrate a delay in feeding behaviour as a potential indicator of pain perception in snakes, and future work investigating the efficacy of analgesia using this model shows promise.

## A9.13 MECHANISMS OF PREDATOR-INDUCED PHENOTYPIC PLASTICITY IN THE FRESHWATER CRUSTACEAN DAPHNIA

WEDNESDAY 6 JULY, 2016 14:35

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The freshwater crustacean *Daphnia* is famous for its high degree of phenotypic plasticity. Based on the same genotype *Daphnia* adapts its phenotype upon changing environmental conditions. E.g. *Daphnia* develops defensive traits such as thorns, elongated spines, neck teeth, helmets and crests upon the perception of predator specific chemical cues. To understand the basis of phenotypic plasticity, the description of the precedent cellular and neuronal mechanisms is fundamental. However, the perceptive organ and the underlying signalling pathways have been left undetermined. We here present the progress that has been made in the identification of perceptive organs and key regulators of the signalling cascade underlying predator induced plasticity in *Daphnia*. We show that *Daphnia* perceive their predators with the antennule and process

this information in the central nervous system via cholinergic and dopaminergic signalling. Based on immunohistochemistry, transcriptional profiling and physiological assays, we developed a conceptual network giving a first insight into the signalling cascade underlying predator induced morphological defences.

## A9.14 PREDATION RISK AND PARENTAL EFFECTS INFLUENCE TOXIN CONTENT AND COLOURATION OF LADYBIRD EGGS

WEDNESDAY 6 JULY, 2016 14:50

SARAH C PAUL (EXETER UNIVERSITY, UNITED KINGDOM), MIKE BIRKETT (ROTHAMSTED RESEARCH, UNITED KINGDOM), MARTIN STEVENS (UNIVERSITY OF EXETER, UNITED KINGDOM), JONATHAN D BLOUNT (UNIVERSITY OF EXETER, UNITED KINGDOM)

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In species that colourfully advertise their toxicity to predators, there is considerable variation between individuals in both signal appearance and levels of defence. Parental effects, i.e. non-genetic inheritance, may play a key role in creating and maintaining this diversity, however a comprehensive test of this notion is lacking. Using the ladybird *Adalia bipunctata* we assess whether egg colouration and toxin level (concentration of the toxic alkaloid adaline), is influenced by maternally detected changes in offspring predation risk, whilst also considering the effect of parental phenotype. We show that that egg colouration, but not egg toxin level, varies between predator treatments, and that the direction of this change is dependent upon predator species identity. Egg luminance decreases in response to conspecific but not heterospecific predation risk, while conversely egg saturation increases in response to heterospecific but not conspecific predation risk. Furthermore, maternal toxin level and paternal elytral colouration positively predicted egg toxin level and egg colouration, respectively. This study provides the first demonstration of maternally mediated offspring colour change in response to predation risk and highlights the importance of studying multiple non-genetic parental effects in determining offspring phenotype.

## A9.15 SIMILAR BURROW ARCHITECTURE IN THREE SCORPION SPECIES IMPLIES SIMILAR ECOLOGICAL FUNCTION

WEDNESDAY 6 JULY, 2016 15:05

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Burrows serve as refuges from predators and adverse environmental conditions for animals ranging in size from ants to aardvarks. Burrow design varies widely among and within taxa, but these structures must be adaptive, fulfilling physiological (and other) functions.

We examined the burrow architecture of three scorpion species of the family Scorpionidae: *Scorpio palmatus* from the Negev Desert, Israel; *Opisthophthalmus setifrons*, from the Central Highlands, Namibia; and *O. wahlbergii* from the Kalahari Desert, Namibia. We hypothesized that burrow structure maintains temperature and soil moisture conditions optimal for the behavior and physiology of the scorpion. Casts of burrows, poured in situ with molten aluminum, were scanned in 3D to quantify burrow structure. Three architectural features were common to the burrows of all species: 1) a horizontal platform near the ground surface, long enough to accommodate the scorpion, located just below the entrance, 2-5 cm under the surface. The entrance platform may provide a safe place where the scorpion can monitor the presence of potential prey, predators, and mates, and where the scorpion warms up before foraging; 2) at least two bends that might deter incursion by predators and may reduce convective ventilation, thereby maintaining relatively high humidity and low temperature; and 3) an enlarged terminal chamber to a depth at which temperatures are almost constant (2-4 °C). These common features among the burrows of three different species suggest they are important for regulating the physical environment of their inhabitants, and that burrows are part of scorpions' "extended physiology" (sensu Turner 2000).

### A9.16 INTERACTIONS BETWEEN PARENTAL TRAITS, ENVIRONMENTAL HARSHNESS AND GROWTH RATE IN DETERMINING RATES OF TELOMERE LOSS IN WILD JUVENILE SALMON

WEDNESDAY 6 JULY, 2016 15:45

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A larger body size has many benefits, such as increased reproductive success, ability to evade predators and increased competitive ability and social status. Attaining a large size requires either prolonged or faster growth, however there is evidence that individuals rarely maximise their growth rates, suggesting that there are costs associated with rapid growth.

Telomere length might be a good indicator of these costs. Telomeres cap the ends of eukaryotic chromosomes and play an important role in chromosome protection. Telomere loss occurs naturally at each cell division and is therefore also associated with growth. Telomere loss may also be accelerated by environmental stressors, such as the production of reactive oxygen species (ROS). A relatively short telomere length is indicative of poor biological state e.g. impending senescence, reduced potential longevity and increased disease susceptibility.

Our study demonstrates the complexity of the environmental factors that can influence telomere dynamics in early life. Using a wild system involving experimental manipulations of Atlantic salmon fry in Scottish streams, we found that both offspring telomere length and rate of telomere loss are influenced by various parental traits and by direct environmental effects. We found that naturally-induced variation in growth rate had a significant effect on fry telomere length. However the rate of telomere loss per unit of

growth was dependent on whether the fry were living in a harsh or a benign environment. This suggests that there may be long term consequences of growth conditions for individual longevity.

### A9.17 DAILY CYCLIC HYPOXIA INDUCES THE MOULT CYCLE IN THE SHRIMP *PALAEMON VARIANS*: CLUES FROM A TRANSCRIPTOMIC APPROACH

WEDNESDAY 6 JULY, 2016 16:00

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Oxygen content in the oceans is declining, but evidence suggests that this decline is more prevalent along the 30 km band near the coast. In shallow-water areas the resultant hypoxia is increasing in frequency and is considered a major threat to biota because it impairs biogeochemical processes at species level, which are suggested to translate into changes in biodiversity and ecosystem functioning. To determine the ecophysiological implications of hypoxia on shallow-water crustaceans, we performed a 30-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*. Using *de novo* assembled shrimp transcriptomes, we have identified significant changes in the expression of key metabolic enzymes, like Glucose-6-phosphate translocase that catalyses the terminal reactions in both glycogenolysis and gluconeogenesis, and Apolipoprotein-II that shuttles lipids between tissues, and in moult-related gene expression, which has never been described in crustaceans before. A general up-regulation of cuticular proteins and chitinases was found, in addition to post-moult stage specific proteins, namely Post-Moult Protein, Calcification Associated Peptide, Gastrolith Protein, and the exoskeletal protein DD5. To further validate the data, we conducted an experiment to determine changes in the duration of the moult cycle. Results clearly support changes to the regulation and duration of the moult cycle, which is accelerated in response to hypoxia. We discuss the observed changes of individual growth and fitness in response to daily oxygen fluctuations, as well as the resulting ecological consequences for the species.

## A9.18 HYPOXIA TOLERANT SPECIES TAKE ADVANTAGE OF INTRACELLULAR ACIDOSIS TO MAINTAIN MITOCHONDRIAL FUNCTION

WEDNESDAY 6 JULY, 2016 16:15

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Fish inhabit environments with variable oxygen supplies, in particular for intertidal fishes. Problematically, hypoxia promotes anaerobic metabolism, lactate accumulation and associated cellular acidosis. The brain is very sensitive to the accumulation of protons, and this likely impacts hypoxic brain mitochondria (mt). While mt consume oxygen ( $\text{JO}_2$ ) to generate chemical ( $\Delta\text{pH}$ ) and electrical ( $\Delta\psi$ ) gradients across the inner-mt-membranes to produce ATP, the effects of extramitochondrial pH on brain mt function remains largely unexplored. We predicted that hypoxia-tolerant species (HTS) should better tolerate acidosis than hypoxia-sensitive species (HSS) in terms of buffering capacities and mt function. Using high resolution respirometry we titrated lactic-acid to decrease extramitochondrial pH, and simultaneously follow  $\text{JO}_2$ ,  $\Delta\psi$  and  $\text{H}^+$  buffering capacities of brain mt within permeabilised brain. Four New Zealand triplefin fish species were compared, each with different hypoxia-tolerances and ranging from high intertidal to subtidal niches. While HTS and HSS displayed similar  $\text{H}^+$  buffering capacities ( $\sim 5\text{mU pH}\cdot\text{mg}^{-1}$ ), contrasting responses were found for mt function. In HSS 4mM lactate elevated  $\text{JO}_2$ , yet decreased  $\Delta\psi$  by  $\sim 5\%$  with a mild acidosis ( $\Delta\text{pH}-0.3$ ) and mt were totally uncoupled mt at pH 5.8. In contrast, 10mM lactate ( $\Delta\text{pH}-0.6$ ) induced a 15% inhibition of  $\text{JO}_2$  in *Bellapiscus medius*, the most HTS. In *B. medius*  $\Delta\psi$  remained stable and coupling capacity at pH 5.8 was maintained to 30% of that at pH 7.2. Overall, these data indicate that in the HTS *B. medius* decreased pH suppresses  $\text{JO}_2$  yet maintains phosphorylation integrity to extremely low pH.

## A9.19 THE IMPACT OF TEMPERATURE AND OXYGEN ON ISOPOD-MICROBE INTERACTIONS

WEDNESDAY 6 JULY, 2016 16:30

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The interactions between microorganisms and animals are omnipresent in nature and have a significant impact on animal evolution and diversity. The effect of microbes on the host fitness is either positive (mutualism), negative (parasitism) or neutral. Animals may benefit from mutualistic associations by improved

growth and survival, enhanced resistance to pathogens or obtaining essential nutrients. Environmental factors shape mutualistic associations through altering the composition and abundance of gut microbiome. However, it is poorly understood how the interaction between environment and gut microbiome affects host physiology, performance and fitness. We used a two-factorial design to examine the effect of temperature (15°C and 22°C) and oxygen level (10% and 22%) on bacterial and fungal community in the gut of isopod *Porcellio scaber* and to test how microbe community affects growth and survival of the host. Preliminary results reveal that ambient temperature, but not oxygen concentration significantly affects bacterial gut diversity. Individuals maintained in warm temperature showed higher bacterial gut diversity than individuals in cold temperature. In the next step, we will analyse community composition of gut fungi and bacteria in order to link the environmental heterogeneity with isopod-microbe interactions. This study will provide novel insights in how variation in life-history traits can be explained by the synergistic action of abiotic and biotic factors.

## A9.20 CORAL REEF FISHES SHOW NEGLIGIBLE PHYSIOLOGICAL AND BEHAVIOURAL ADJUSTMENTS TO ELEVATED $\text{CO}_2$

WEDNESDAY 6 JULY, 2016 16:45

JOSEFIN SUNDIN (UPPSALA UNIVERSITY, SWEDEN), MIRJAM AMCOFF (UNIVERSITY OF TEXAS, UNITED STATES), FERNANDO MATEOS-GONZÁLEZ (UNIVERSITY OF KONSTANZ, GERMANY), GRAHAM D RABY (UNIVERSITY OF WINDSOR, CANADA), FREDRIK JUTFELT (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY), TIMOTHY D CLARK (UNIVERSITY OF TASMANIA AND CSIRO AGRICULTURE FLAGSHIP, AUSTRALIA)

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Much of the anthropogenically-released carbon dioxide dissolves in the ocean, causing ocean acidification (OA). Exposure to elevated  $\text{CO}_2$  levels has been reported to effect physiology and behaviour of fishes, which could have detrimental consequences for population viability in the future. However, a growing number of studies report no physiological or behavioural changes, suggesting a far from complete understanding of the potential effects of OA. We investigated the possible effect of both short- and long-term exposure to  $\text{CO}_2$  on physiology and behaviour, using several different species of wild-caught and laboratory-raised coral reef fishes. We found that all species were resilient to  $\text{CO}_2$  exposure, as we were unable to replicate the dramatic impairments previously reported, and we found no evidence for interference of  $\text{GABA}_A$  neurotransmitter function. Our findings highlight the need for independent replication before we can reach a consensus on the ecological effects of OA.

## A9.21 ADAPTIVE RESPONSE OF A SEA URCHIN *PARACENTROTUS LIVIDUS* POPULATION INHABITING VOLCANIC CO<sub>2</sub> VENTS (TYRRHENIAN SEA, ITALY)

WEDNESDAY 6 JULY, 2016 17:00

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Ocean acidification (OA) has been recognized as an emerging global stressor, potentially affecting ecosystems' biodiversity, functions and services. Studies on the effects of OA on marine organisms have been primarily conducted in laboratory, thus preventing the prediction of long-term consequences in naturally multi-stressed environments. In this study we investigated the effects of near-future OA on *Paracentrotus lividus* inhabiting shallow-water volcanic CO<sub>2</sub> vents, an established naturally acidified site that offers precious opportunity to investigate long-term and/or adaptive responses of species to OA. Sea urchin persistence in the moderately acidified areas (pH~7.8) was monitored in situ for few months by using non-destructive tagging techniques. Animals were examined by measuring morphometric parameters, routine metabolism, nitrogen excretion rates, along with biochemical analysis of gonads, immune cells and coelomic fluids. Our data indicated that the *P. lividus* population permanently inhabits CO<sub>2</sub> vents. Animals showed a mean size similar to those collected at control sites, suggesting that low pH/high pCO<sub>2</sub> conditions do not affect their growth rate. Our results indicated that population at CO<sub>2</sub> vents does not face stressful conditions, as revealed by the measurements of lipid peroxidation, nitrite and hsp70 protein levels along with the determination of type/number of immune cells. Nevertheless, changes in coelomic fluid composition, together with an increased total antioxidant capacity, indicated the occurrence of adaptation processes in animals inhabiting the acidified site. In conclusion, we suggest that OA does not represent a forthcoming threat for *P. lividus* since the animals show a great potential of adaptation to near-future OA conditions.

## A9.22 RUDDY SHELDUCK: A HIGH ALTITUDE MIGRANT

WEDNESDAY 6 JULY, 2016 POSTER SESSION

NICOLE PARR (UNIVERSITY OF EXETER, UNITED KINGDOM), DAVID DOUGLAS (USGS, UNITED STATES), SCOTT NEWMAN (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO), UNITED STATES), WILLIAM PERRY (USGS, UNITED STATES), DIANN PROSSER (USGS, UNITED STATES), JOHN Y TAKEKAWA (USGS, UNITED STATES), LUCY HAWKES (UNIVERSITY OF EXETER, UNITED KINGDOM)

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High altitude environments pose a number of challenges to humans and animals including greatly reduced oxygen availability. Despite this, birds are known to make migrations across mountain ranges, encountering reduced partial pressures of oxygen, while engaging in one of the most costly forms of locomotion in terms of oxygen requirement (flight). In order to investigate the limitations of hypoxia tolerance in a high altitude migrant, we used satellite tracking to record the migration of 15 Ruddy Shelduck as they flew from wintering grounds in Southern Asia, to breeding grounds in central China, including their flight across the eastern Himalayan Mountain ranges. During these flights we recorded Ruddy Shelduck flying up to 6800m, with a median climb rate of ascending birds of 203.4 m hour<sup>-1</sup> (range 2.5-3098 m hour<sup>-1</sup>). These findings are discussed in terms of the cost of sustaining flapping and intermittent climbing flight in conditions where the oxygen content is as little as half that at sea level. Furthermore, these findings suggest that bar headed geese may not be the only migrating bird showcasing numerous physiological adaptations to help meet the increased costs of flight at high altitude.

## A9.23 EFFECTS OF AN ECOSYSTEM ENGINEER MOTH IN A TROPICAL ENVIRONMENT: INCREASE OF ABUNDANCE AND DIVERSITY OF SPECIES ASSOCIATED TO THE HOST PLANT

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Variation in plant phenology allows plants to escape from herbivory. Insect herbivores manipulate their host plants by producing shelters, which they inhabit, and are protected against natural enemies and/or unfavorable environmental conditions. Environmental modifications induced by living organisms are characterised as ecosystem engineering. We studied the interaction between a Malpighiaceae shrub, *Byrsonima intermedia*, and its main herbivore, the caterpillar *Cerconota achatina*, a shelter-building organism in the Brazilian savanna. The environment was very seasonally inconstant, particularly regarding rainfall, which regulated several aspects of *B. intermedia*. We focused on whether the phenological development of the host plant affects the infestation and success of the caterpillars, and whether *C. achatina* acts as an ecosystem

engineer by building shelters. All plant variables (number of leaves, flowers, buds, fruits and herbivores) were measured fortnightly. Phenological data were correlated with climatic information. The impact of the caterpillars acting as ecosystem engineers was measured experimentally. *Cerconota achatina* acts as a true ecosystem engineer, increasing the diversity and abundance of species associated with *B. intermedia* in both dry and wet seasons. This study is the first to quantify the effect of an ecosystem engineer in a tropical environment with strong variations in seasonality and plant phenology.

### A9.24 RELATIONSHIP BETWEEN AGGREGATIONS OF A SOLITARY BEE AND ATTRACTIVENESS OF NESTING PLACE FOR OTHER SPECIES OF TRAP-NESTING INSECTS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Many species of solitary Hymenoptera nest in aggregations. Aggregated nesting may be favoured because of presence of conspecifics, or it is only a by-product of choosing suitable environmental conditions by many individuals. One of the hypotheses explaining preference of aggregations postulates more effective protection against nesting parasites in larger aggregations. Because many nesting parasites attack several species, we hypothesized that nesting in heterospecific aggregations may protect nests of all aggregation members against parasites. Then, females should favour nesting in existing aggregations even if they consist of other species. In our experiment we aimed to check whether an existing aggregation of one species of solitary Hymenoptera will attract other species to establish nests there. We compared abundance of solitary Hymenoptera nesting in artificial trapnests (consisting of reed straws) with pre-established red mason bee aggregations, or without them. Contrary to our expectations, the numbers of nests established by other species in trapnests with or without red mason bee aggregation did not differ significantly, and the risk of parasitism also did not differ between the trapnests. Our results suggest that females in our experiment were attracted to the presence of nesting material (which in natural conditions tends to be a limiting factor), and presence of heterospecific aggregation did not play an important role in selection of nesting place.

### A9.25 INTER- VS INTRA-INDIVIDUAL VARIATION AND TEMPORAL REPEATABILITY OF ESCAPE RESPONSES IN THE CORAL REEF FISH *AMBLYGLYPHIDODON CURACAO*

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Fast-start escape responses are critical behaviours used by fishes during predator-prey encounters and some interactions with hetero- and conspecifics. In experimental studies, escape responses are often measured once per individual and considered representative of maximum performance. However, few studies have compared variability and repeatability in escape performances within and among individuals. Using the tropical damselfish *Amblyglyphidodon curacao*, we quantified inter- and intra-individual variation in behavioural and kinematic components of escape performance during repeated presentations of a stimulus at 15 min intervals. Individual maximum escape performance was repeatable through time, but there was considerable variation in the magnitude of responses both among and within fish. We found no evidence of habituation or fatigue due to repeated stimulations, suggesting that fish can be stimulated multiple times to ensure that an accurate estimate of maximum escape performance is obtained.

### A9.26 THE EFFECT OF TEMPERATURE ACCLIMATION ON TRANSCRIPTION IN RAINBOW TROUT GILL CELLS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Climate change is most evident at higher latitudes, where daily and seasonal changes in temperature are pronounced. Cells of poikilotherms exhibit the ability to acclimate to different temperatures and to understand how fish are able to adapt to a warming environment, we wanted to evaluate if gene transcription rates also exhibit acclimatory responses. The nuclear run-on assay provides an accurate quantification of transcription rates, however the temperature at which transcription rate is measured is usually performed at a temperature considerably higher than what temperate fish experience in nature. Thus, the aim of the study is to determine how transcription rates of metabolic enzyme genes are affected by the different in-vitro reaction temperatures and how the transcription of glycolytic, anaerobic and aerobic enzyme genes are altered by acclimation to different growth temperatures.

## A9.27 ONSET OF KAIROMONE SENSITIVITY AND THE DEVELOPMENT OF INDUCIBLE MORPHOLOGICAL DEFENCES IN *DAPHNIA PULEX*

WEDNESDAY 6 JULY, 2016 POSTER SESSION

• SINA M BECKER (RUHR-UNIVERSITY BOCHUM, GERMANY), ESTHER HEILGENBERG (RUHR-UNIVERSITY BOCHUM, GERMANY), LISA DEUSSEN (RUHR-UNIVERSITY BOCHUM, GERMANY), SEBASTIAN KRUPPERT (RUHR-UNIVERSITY BOCHUM, GERMANY), RALPH TOLLRIAN (RUHR-UNIVERSITY BOCHUM, GERMANY), LINDA C WEISS (RUHR-UNIVERSITY BOCHUM, GERMANY)

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The micro-crustacean *Daphnia pulex* is a model species for studying predator-induced defences. When exposed to chemical cues released by its predator, the phantom midge larvae *Chaoborus* (Diptera), it develops protective neckteeth that reduce the predator's success of predation in the juvenile instars. Defensive traits need to be expressed as soon as possible, which requires an early sensitivity to predator cues. We investigated the exact kairomone sensitive period in three *Daphnia pulex* strains and the timeline of neckteeth expression in early juvenile instars. For that, we divided embryonic development into five major stages based on successive morphological landmarks. We stimulated these stages during different time points and intervals to determine the sensitive periods for neckteeth expression in the 1<sup>st</sup> and 2<sup>nd</sup> juvenile instar. Our results indicate that kairomone sensitivity starts during embryogenesis when compound eyespots begin to fuse and egg membranes are shed. Neckteeth develop with a stage dependent time lag, being shorter when exposed in the first kairomone sensitive stage and longer when exposed in the following developmental stages. Evolution of early kairomone sensitivity and fast defence development is a crucial step in *D. pulex*'s defences against *Chaoborus* as it allows for protection of the most vulnerable juvenile stages.

## A9.28 DEVELOPMENT AND ADAPTATION OF AN *IN VITRO* RAINBOW TROUT GILL MODEL FOR USE AS AN ALTERNATIVE TO LIVE FISH STUDIES

WEDNESDAY 6 JULY, 2016 POSTER SESSION

• RICHARD J MAUNDER (PLYMOUTH UNIVERSITY, UNITED KINGDOM), MATTHEW G BARON (PLYMOUTH UNIVERSITY, UNITED KINGDOM), STEWART F OWEN (GLOBAL SAFETY HEALTH AND ENVIRONMENT ASTRAZENCA, UNITED KINGDOM), AWADHESH N JHA (PLYMOUTH UNIVERSITY, UNITED KINGDOM)

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The development and validation of reliable *in vitro* methods that offer an alternative to conventional *in vivo* studies is becoming increasingly important. We have recently initiated methods of primary cell culture for different cell types of rainbow trout (liver, gut and gills), and aim to combine these tissues in co-cultures to provide an *in vitro* model with a higher degree of predictivity towards *in vivo* responses. Here we report on work investigating the further development and adaptation of an existing double-seeded gill epithelial model that is grown on a cell culture insert within a

microplate well. The model is a humane alternative to *in vivo* studies of gill physiology, toxicity testing, bioaccumulation studies and water quality monitoring. The study aims were to investigate the effect of different culture methods on the gill model viability and to maximise its lifespan and functionality. We found that the time taken to produce the cultures could be reduced by removing blood cells from the gills via perfusion prior to use. Culture assessment via trans-epithelial resistance and scanning electron microscopy revealed that cells did not adhere to membranes with higher porosity, but that time to confluence and response to apical water addition could be improved by supplementing with native serum and by growing under rotational flow. Indeed, gills from larger fish (~400g) could be cultured successfully and the fish provide their own serum for later culture. Future work aims to further characterise this robust model and use within toxicological and physiological research.

## A9.29 WARMING AND COOLING RATES ARE UNAFFECTED BY AUTONOMIC VASCULAR CONTROL IN THE SOUTH AMERICAN RATTLESNAKE

WEDNESDAY 6 JULY, 2016 POSTER SESSION

• RENATO FILOGONIO (AARHUS UNIVERSITY, DENMARK), CLÉO A. C. LEITE (UFSCAR, BRAZIL), AUGUSTO S. ABE (UNESP-RIO CLARO, BRAZIL), TOBIAS WANG (AARHUS UNIVERSITY, DENMARK)

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Reptiles typically heat faster than they cool. Resulting thermal hysteresis is believed to stem from increased skin perfusion during heating, whilst cutaneous perfusion is reduced during cooling to retain body heat. Here,  $\alpha$ -adrenergic control of the vasculature is believed to be of paramount importance, and hence reflecting a sympathetic regulation of thermal conductance over the skin. It has also been proposed that vagal control over pulmonary blood flow serves to shunt blood away from pulmonary circulation, avoiding heat dispersal through respiratory surface. In the South American rattlesnake (*Crotalus durissus*), parasympathetic control of pulmonary vascular resistance is solely under regulation of the left vagus, such that left vagotomy results in loss of cardiac shunt control without disrupting other autonomic functions. We tested the influence of the aforementioned mechanisms on warming and cooling rates of *C. durissus* by implanting temperature loggers on 11 snakes – separated into a control and a left vagotomised group – and injecting them with  $\alpha$ -adrenergic blocker phentolamine. Snakes were free to thermoregulate within a climatic chamber with constant 15°C air temperature and intermittent heating source (on: off - 12:12h). Snakes warmed faster ( $1.2 \pm 0.9$  min/°C) than cooled ( $12 \pm 1.4$  min/°C), and behavioral thermoregulation was apparent whenever body temperature reached ~25°C. None of them stabilized body temperature with room temperature during cooling. No differences in warming and cooling rates were observed between treatments with the  $\alpha$ -blockade or vagotomisation. These results indicate that physiological thermoregulation in cylindrical low mass reptiles is unaffected by autonomic vascular regulation, and question the functional significance of cardiac shunts.

### A9.30 PHYSIOLOGICAL AND BEHAVIOURAL RESPONSES UNDER HYPOXIC CONDITIONS IN INVASIVE AND NATIVE FRESHWATER FISH SPECIES

WEDNESDAY 6 JULY, 2016 POSTER SESSION

JULIE JH NATI (UNIVERSITY OF GLASGOW, UNITED KINGDOM), JAN LINDSTRÖM (UNIVERSITY OF GLASGOW, UNITED KINGDOM), WILLIAM YEOMANS (UNIVERSITY OF GLASGOW, UNITED KINGDOM), SHAUN S KILLEN (UNIVERSITY OF GLASGOW, UNITED KINGDOM)

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The spread of non-native fish species into novel environments is predicted to increase with climate change. While some introduced species have minimal effects on their new habitats, others become invaders with a number of severe impacts on ecosystems, often causing displacement of native fish species. The mechanisms that allow certain species to become successful invaders are still poorly understood, but one characteristic defining a good invader is the capacity to cope and thrive in disturbed environments. In the events of unfavourable conditions, such as hypoxic episodes, invasive species might be better adapted and flexible in their physiological and behavioural responses towards this stressor. In Scotland, the bullhead (*Cottus gobio*) is considered invasive after being introduced in the Clyde River 100 years ago. This freshwater fish species is suspected to coexist and compete with the native benthic stone loach (*Barbatula barbatula*) within the same ecological niche. In this study, we compared physiological (e.g. metabolic rate) and behavioural traits between bullheads and stone loaches over different dissolved oxygen (DO) concentrations in the water (100%, 80%, 60%, 40%, 30%, 25% and 20% DO) with the aim to investigate and compare the coping capacities between the two fish species facing hypoxic conditions.

### A9.31 ELUCIDATION OF FINE-SCALE MOVEMENT BEHAVIOUR IN EUROPEAN BADGERS (*MELES MELES*)

WEDNESDAY 6 JULY, 2016 POSTER SESSION

KATIE BARBOUR (QUEENS UNIVERSITY BELFAST, UNITED KINGDOM), NIKKI J MARKS (QUEENS UNIVERSITY BELFAST, UNITED KINGDOM), RICHARD J DELAHAY (NATIONAL WILDLIFE MANAGEMENT CENTRE ANIMAL AND PLANT HEALTH AGENCY, UNITED KINGDOM), RORY P WILSON (SWANSEA UNIVERSITY, UNITED KINGDOM), MIKE SCANTLEBURY (QUEENS UNIVERSITY BELFAST, UNITED KINGDOM)

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Studies describing the movement of free-ranging animals have recently become heavily reliant on the use of remotely collected global positioning system (GPS) data. However, these data typically only include intermittent positional information, with a sampling frequency that is constrained by battery life. 'Dead reckoning' of animal movements, which uses the combined information from GPS and tri-axial accelerometer and magnetometer loggers is one alternative. This approach has the potential to provide continuous information on animal movement, behaviour and interactions

with various habitat features, providing important ecological information on animal-environment interactions. We examine movement patterns of the European badger, a species otherwise difficult to study because of its nocturnal and fossorial lifestyle. Home range sizes and distances travelled are compared between GPS and GPS-enhanced dead reckoned tracks to highlight the additional information that can be gathered using this technique. Initial results suggest the dead-reckoned distance travelled per night is 1.5 times greater than distance travelled per night estimated via GPS.

### A9.32 AUTONOMIC REGULATION AND THE ROLE OF NITRIC OXIDE ON THE CIRCULATION OF THE AFRICAN LUNGFISH, *PROTOPTERUS AETHIOPICUS*

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Lungfishes are key organisms to understand the transition from water to land amongst vertebrates. Unlike other fish, lungfishes are endowed with both systemic and pulmonary circulations, and their incompletely divided ventricle allows for blood to bypass either circuit depending on differences between vascular conductances. To understand how vascular conductances are affected by autonomic nervous system and nitric oxide (NO) in African lungfishes (*Protopterus aethiopicus*), we measured vascular reactivity of four vessel segments *in vitro*: efferent branchial arteries, gill artery, ductus arteriosus and pulmonary artery. Dose-response curves were constructed using increasing doses of the muscarinic agonist acetylcholine,  $\alpha$  and  $\beta$ -adrenergic agonists (phenylephrine and isoproterenol, respectively), or the NO donor, sodium nitroprusside (SNP). Only ductus arteriosus vasodilated in response to SNP, with a maximum effective active pressure ( $\Delta p$ ) of  $0.26 \pm 0.15$  kPa, but was unaffected by NO precursor, L-arginine. Isoproterenol caused vasodilation in all segments tested, although weaker than SNP at the ductus arteriosus ( $\Delta p = 0.06 \psi 0.03$  kPa). At higher concentrations, isoproterenol caused vasoconstriction that returned values close to baseline, which was blocked by  $\alpha$ -adrenergic antagonist phentolamine. Acetylcholine caused vasoconstriction in all segments, particularly in the pulmonary artery ( $\Delta p = 1.5 \pm 0.3$  kPa). All vessels vasoconstricted with phenylephrine, but pulmonary artery's response was weaker ( $\Delta p = 0.5 \psi 0.1$  kPa) and at higher doses than acetylcholine. Given that L-arginine did not stimulate endothelial NO synthesis, our results indicate that NO is derived from perivascular nitrergic nerves on the ductus arteriosus. Additionally, muscarinic stimulation apparently has a stronger effect on pulmonary vasculature than adrenergic stimulation in lungfishes.

### A9.33 BIRDS RECOGNIZE PARASITIC EGGS USING COLOUR CATEGORIZATION

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Hosts of avian brood parasites must recognize and reject parasitic eggs or suffer the fitness consequences of raising an unrelated offspring. Egg discrimination is a difficult cognitive task, and scientists have long assumed that hosts reject foreign eggs based on their degree of perceived colour dissimilarity to their own eggs. However, it is also possible that hosts use more complex cognitive decision-rules, e.g., colour categorization, to make rejection decisions. Here, through a series of experiments, we explored host responses across the avian perceived colour space. We found that hosts rejected brown eggs but accepted equally dissimilar blue-green eggs, which suggests that hosts use colour categorization for rejection decisions rather than basing decisions on perceived colour differences. These findings provide an important advance in our understanding of host cognitive mechanisms, and provide novel directions for research in the area of host-brood parasite coevolution.

### A9.34 ANALYSIS OF ENERGY DENSITY VARIATIONS IN SARDINE (*SARDINA PILCHARDUS*) IN THE ENGLISH CHANNEL AND THE BAY OF BISCAY

WEDNESDAY 6 JULY, 2016 POSTER SESSION

LOUISE COMINASSI (UNIVERSITY OF HAMBURG, GERMANY), MARTIN HURET (IFREMER, FRANCE), PAUL GATTI (IFREMER, FRANCE), HERVÉ LE DELLIUOX (IFREMER, FRANCE), ESTELLE MONGES (IUEM, FRANCE)

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The pressures induced by climate change and human activities lead to a decrease in fish stocks abundance and may also alter their movement and/or behavior. To understand and grasp these changes it is possible to study the spatial and temporal evolution of fish using condition index such as the energy density (ED).

The energy density of the sardine (*Sardina pilchardus*) was determined, in the English Channel and in the Bay of Biscay, by direct calorimetry. First, two parameters, the index of Fulton (K) and humidity, which are used to estimate the energy of fish, have been correlated with ED. The index of Fulton (K) is correlated with ED as humidity; however this parameter follows the opposite trend. The index K masks the loss of water which takes place in winter, as such this index is less informative than ED. Secondly, the variability of ED was investigated depending on size, season and space. The results obtained show that ED increases with size. Furthermore, ED of dry mass ( $M_{dry}$ ) vary significantly during the year with high values in autumn (28.8 kJ.g<sup>-1</sup>) and lowest in winter (19.6 kJ.g<sup>-1</sup>). During winter reserves diminish in favor of water content. These variations of organic matter and water content can be related to the availability of food and the reproductive cycle. ED variations in sardines were also

studied spatially. Thus, a spatial variation was observed between the English Channel and the Bay of Biscay in fall and in the Bay of Biscay in spring.

### A9.35 RESTORED PHENOTYPES BY ATG8 COMPLEMENTATION

WEDNESDAY 6 JULY, 2016 POSTER SESSION

ALANNA C. F. PEREIRA (UNIVERSIDADE ESTADUAL DE SANTA CRUZ, BRAZIL), FABIANA A. C. SILVA (UNIVERSIDADE ESTADUAL DE SANTA CRUZ, BRAZIL), MARTIM BRENDEL (UNIVERSIDADE ESTADUAL DE SANTA CRUZ, BRAZIL), CRISTINA PUNGARTNIK (UNIVERSIDADE ESTADUAL DE SANTA CRUZ, BRAZIL)

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Autophagy (ATG) is a cellular process that causes degradation of long-lived proteins and recycling of cellular components to assure survival during periods of nutritional lack or other environmental stresses. In this process *Atg8* protein is essential for formation of the system. The role of the secretory pathway in autophagy is largely by studies in yeast, the importance of the autophagy process can be verified in mutants *atg8Δ*; the *S. cerevisiae* for saw present characterized phenotype already. *M. perniciosus* putative autophagy gene *MpATG8* was tested by introducing it into yeast mutant *atg8Δ*; and testing for heterologous expression phenotypic sporulation complementation and *TcPR-10p* sensitivity, the pathogenesis-related protein PR-10 of *Theobroma cacao* has antifungal action and ribonuclease activity *in vitro*. Formation of oxygen radicals (ROS) after exposure to *TcPR-10p* was observed using fluorescence microscopy with dihydroethidium-stained cells. WT and mutant *atg8Δ*; transformed with a single-copy vector containing *MpATG8* gene showed practically the same resistance to *TcPR-10p* and similar formation of ROS, while mutant *atg8Δ*; was sensitive and exhibited increased ROS accumulation. This suggests that the protein codified by *MpATG8* is functionally expressed in *S. cerevisiae* and protects against *TcPR-10p* whereas mutant *atg8Δ* accumulates ROS under the same conditions, also our results show the sporulation could be restored in *atg8Δ/atg8Δ* diploids when transformed with one copy of *MpATG8*.

### A9.36 THE ORIGIN OF MASS-SCALING OF METABOLIC RATE & TESTING THE ROLE OF BODY COMPOSITION AND CELL SIZE IN CARABIDAE BEETLES

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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A decelerating increase of metabolic rate with body mass is one of the most notable, yet still poorly understood patterns in biology. To describe this phenomenon, the dynamic energy budget (DEB) hypothesis predicts a disproportionate increase in mass of the metabolically inactive tissues (e.g. skeletal mass and reserve fats) with body mass. Conversely, optimal cell size theory proposes the relationship between cell size and number as the driving force for the variations in the scaling of respiration rate with body mass. Specifically, it postulates the decrease in membrane area to cell volume ratio, which in turn would result in lower maintenance costs of the cells themselves.

Here, we studied phylogenetically corrected metabolic rate, body composition, cell size (epithelium cells of Malpighian tubules), and a cell size proxy (ommatidia facet size) in 15 species of Carabidae beetles testing the predictions of the aforementioned hypotheses. Body composition proved to be inadequate to explain the negative allometry of respiration rate predicted by the DEB. Exoskeletal mass scaled with a negative allometry ( $p < 0.001$ ) with tissue mass whilst the fat reserves were body mass-invariant with a scaling exponent equal to 1. Simultaneously, a strong positive correlation between cell size and body mass was detected ( $r^2 = 0.56$ ,  $p < 0.01$ ). Additionally, two groups, consisting of more closely related species, exhibited higher respiration rate accompanied by smaller cell size and vice versa. Therefore, we present evidence to support cell size optimization as one of the evolutionary mechanisms underpinning the mass-scaling of metabolism and the variation in this relationship in different taxa.

### A9.37 TWO-CURRENT CHOICE FLUMES FOR FISH CHEMOSENSORY BEHAVIOUR: METHOD VALIDATION AND LIMITED EFFECTS OF HIGH CO<sub>2</sub>

WEDNESDAY 6 JULY, 2016 POSTER SESSION

 JOSEFIN SUNDIN (UPPSALA UNIVERSITY, SWEDEN), FREDRIK JUTFELT (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY), GRAHAM D RABY (UNIVERSITY OF WINDSOR, CANADA), TIMOTHY D CLARK (UNIVERSITY OF TASMANIA AND CSIRO AGRICULTURE FLAGSHIP, AUSTRALIA)

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Ocean acidification has been suggested to disturb fish behavioural responses to chemosensory cues. Choice flumes are often used to assess preference or avoidance of a water source containing specific chemical cues, and a variety of methods have been described in the scientific literature. However, there is a clear absence of standardised methodologies, which makes comparisons across studies difficult. Two-current choice flumes carry two parallel laminar water flows through an arena where the experimental animal can choose between the two flows, and the two flows can be manipulated (e.g. hypoxia, hypercapnia, prey/predatory cues). Here we present 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. We show that high CO<sub>2</sub> appears to have limited effects on temperate as well as coral reef fishes. We encourage the use of these approaches in all future studies to enable a comprehensive and robust understanding of any CO<sub>2</sub> effects on the chemosensory behaviour of fish.

# A10 OPEN ANIMAL BIOLOGY

**ORGANISED BY:** DR PETER HUBBARD (UNIVERSITY OF ALGARVE, PORTUGAL)  
AND DR LYNNE SNEDDON (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM)

## A10.1 SELECTION DRIVES METABOLIC ALLOMETRY

📅 WEDNESDAY 6 JULY, 2016 ⌚ 09:00

👤 CRAIG R WHITE (MONASH UNIVERSITY, AUSTRALIA), DANIEL ORTIZ-BARRIENTOS (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA), DUSTIN J MARSHALL (MONASH UNIVERSITY, AUSTRALIA)

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Living species vary in size from ~0.1 pg single-celled microorganisms to trees weighing several thousands tonnes. Put in perspective, this  $\sim 10^{21}$ -fold range is similar to the difference in mass between an elephant and the Earth itself. The influence of mass on biological processes is pervasive, but is usually allometric: a 10-fold increase in mass is typically accompanied by just a 4-to-7-fold increase in metabolic rate. Understanding the basis of allometric scaling is a long-standing problem in biology. Here, we show the interspecific relationship between metabolic rate and body mass arises as a consequence of correlational selection on these traits, coupled with negative directional selection on absolute metabolic rate. This pattern of selection explains not only the covariance between metabolic rate and body mass (the allometric scaling of metabolic rate), but also explains the magnitude of the conditional variance in metabolic rate. The correlational selection we document constrains the evolution of mass-specific metabolic rates (MSMR) such that the observed range of MSMRs is just 50-fold among species that differ in size by ten billion-fold. Our results link microevolutionary processes to macroevolutionary patterns to describe the evolution of metabolic allometry in animals.

## A10.2 BODY SIZE AND CELL SIZE IN THE NORTH AMERICAN FENCE LIZARD – GEOGRAPHIC PATTERNS IN CLIMATE, PHYLOGENY, BODY SIZE AND ERYTHROCYTES SIZE

📅 WEDNESDAY 6 JULY, 2016 ⌚ 09:15

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Body size affects vital organismal traits such as metabolic rate, fecundity or survival. A change in body size results from a change in cell number and/or cell size, and the theory of optimal cell size predicts that cell size itself is the target of selection. On the one hand, small cells provide relatively large membrane area for oxygen and nutrients transport which can help ectotherms to meet increased demand for resources in warm or thermally fluctuating environments. On the other hand, large amount of membranes require more resources to maintain their physiological function, what makes large cells beneficial in resource-deficient environments. We analyzed body size and erythrocytes size (our proxy of cell size) in the fence lizard *Sceloporus undulatus*. The studied lizards originated from eight populations with known phylogenetic relationships, located at different latitudes and longitudes in North America. Northern lizards had generally larger body size and smaller erythrocytes than southern lizards, though clades differed with respect to this latitudinal pattern as well as the absolute values of the studied traits. The results are discussed with reference to data on local microclimates, phylogeny and life history.

### A10.3 EFFECT OF PERFORMANCE-BASED PHYSICAL ACTIVITY ON THE PLASMA PROTEOME OF BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*)

WEDNESDAY 6 JULY, 2016 09:30

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This study was designed to characterize the plasma proteome and its response to short-term physical activity in bottlenose dolphins (*Tursiops truncatus*). Blood samples were collected from the tail flukes of four male dolphins (2 to 6 years) housed at Dolphin Discovery in St. Kitts (West Indies). Collections were made in tubes containing sodium citrate and were done within 15 min before and 15 min after performance-based physical activity. Physical activity was defined as an approximately 44 min long swim interaction with the public. For each of the eight samples, 50 µg of proteins was extracted from 200 µL of plasma and were digested using trypsin for shot-gun proteomics. Peptides were analysed by liquid chromatography-mass spectrometry (LC-MS) for identification and quantification of label-free proteins. Mass spectra were searched against NCBI and SwissProt databases. This resulted in the identification of 226 unique proteins with at least 2 peptides, which were subjected to manual gene ontology analysis. Of total proteins, protein binding was most prevalent at 30.5% for molecular function, extracellular region most prevalent at 42.0% for cellular component, and metabolic process was most prevalent at 14.2% for biological process. For protein quantification, an increase or decrease in abundance was based on  $\log_2(\text{mean fold change}) \leq -0.585$  or  $\geq 0.585$ , respectively, with a probability of  $P \leq 0.06$ . The metabolic-related protein flavin reductase (NADPH) and immune-related protein lysozyme f1 decreased following physical activity. Although preliminary and novel, results indicated that the metabolic and immune system pathways were down-regulated in response to short-term physical activity in bottlenose dolphins.

### A10.4 ICCS AND THE CONTROL OF GUT MOTILITY IN SHORTHORN SCULPIN

WEDNESDAY 6 JULY, 2016 09:45

CATHARINA OLSSON (DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES, UNIVERSITY OF GOTHENBURG, SWEDEN), JEROEN BRIJS (DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES, UNIVERSITY OF GOTHENBURG, SWEDEN), GRANT W HENNIG (DEPARTMENT OF PHYSIOLOGY AND CELL BIOLOGY, UNIVERSITY OF RENO, UNITED STATES), ANNA-MARIA KELLERMANN (DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES, UNIVERSITY OF GOTHENBURG, SWEDEN), MICHAEL AXELSSON (DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES, UNIVERSITY OF GOTHENBURG, SWEDEN)

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ICCs, or interstitial cells of Cajal, play a fundamental role in controlling smooth muscle activity in the mammalian gut. By generating spontaneous depolarisations, they act as pacemakers for gut motility and they also convey signals from nerves to muscles. In this study, we investigated the distribution of ICCs along the gastrointestinal tract of shorthorn sculpin (*Myoxocephalus scorpius*) and their involvement in various intestinal motility patterns. Anol1 (anoctamin 1, a known mammalian ICC marker)-immunoreactive ICCs were common in all regions of the gastrointestinal tract and comprised a dense network of multipolar cells between the circular and longitudinal muscle layers. Immunoreactive cells were also seen within the muscle layers. In addition, inhibition of the ICCs altered motility in the proximal intestine as seen using *in vivo* video-recordings. Whereas previous studies have shown that substantial motor activity persists after blocking nervous input with tetrodotoxin (TTX), indicating that nerves are not essential to initiate and maintain gut contractions, the Anol1-blocker benzbramarone nearly abolished all types of motility patterns in the intestine. This indicates that ICCs, similar to what is seen in mammals, are responsible for the so called myogenic activity in fish as well. However, unlike in mammals where ICCs primarily affect the shallow rhythmic propagating contractions referred to as 'ripples', the situation in fish seems to be more complex. Our results indicate that ICCs are involved in both ripples and more slowly anally propagating contractions, i.e. the two major gut motility patterns observed in shorthorn sculpin.

## A10.5 MICROBIAL COMMUNITIES ALONG THE FISH GASTROINTESTINAL TRACT ARE ASSOCIATED WITH VARIOUS PHYSIOLOGICAL PARAMETERS

WEDNESDAY 6 JULY, 2016 10:00

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

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The digestive tract of vertebrate is associated with complex assemblages of microorganisms which are believed to contribute to their host's functioning. Current information on gut microbiota composition and function is derived primarily from mammals, where it has revealed associations between microbial composition and host diet, anatomy and phylogeny. However, information on similar associations in fish is limited. Few studies have shown that dietary ingredients can potentially select for different microbes within the gastrointestinal tract. In addition, fish gastrointestinal tract exhibits regional specialization along the rostral–caudal axis similar to the mammalian gut. Thus, it may be expected that the bacterial taxonomic composition of the various parts in fish would also be different. In the present work, next-generation sequencing was used in order to describe the bacterial community composition along the gut of European sea bass (*Dicentrarchus labrax*). Fish were administered with diets containing different levels of macronutrients. Microbial communities of the main parts of the gastrointestinal tract, pyloric caeca, midgut and hindgut, were characterized by sequencing of the 16S rRNA genes. The results show a unique spatial distribution of the microbiome composition across the gut, which is reinforced by diet and physiological conditions. These findings, suggesting ecological niches across the fish gastrointestinal tract and their functional meaning, will be discussed in the context of host-microbiome interactions. The interpretation of such results is highly crucial for better understanding of the fish gut and microbe interactions.

## A10.6 SYNERGISTIC OR ANTAGONISTIC EFFECTS OF TWO MATERNALLY-DERIVED EGG COMPONENTS (ANTIBODIES AND TESTOSTERONE) ON OFFSPRING PHENOTYPE

WEDNESDAY 6 JULY, 2016 10:15

TONY D WILLIAMS (SIMON FRASER UNIVERSITY, CANADA), EUNICE CHIN (SIMON FRASER UNIVERSITY, CANADA), ROWAN RAMPTON (SIMON FRASER UNIVERSITY, CANADA), ROXANA TORRES (UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO, MEXICO)

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Maternally-derived egg components (non-genetic parental contribution) are thought to modulate offspring development and, potentially, final adult phenotype. Eggs contain multiple maternally derived compounds (e.g. hormones, antibodies,

mRNA, antioxidants) but most studies have focused on single egg components (most often yolk testosterone, or corticosterone), and on short-term effects. Here we simultaneously manipulated two egg components, maternally-derived antibodies (MAB) and yolk testosterone to assess potential synergistic or antagonistic effects on offspring phenotype. We used lipopolysaccharide treatment to generate a secondary immune response in female zebra finches (*Taeniopygia guttata*), which produced clutches of eggs with high (LPS-treated) or low (control) MAB. We then used a split design manipulating yolk testosterone within clutches of high- and low-MAB eggs using in ovo egg injection. We investigated a) short-term effects of experimental manipulation of both egg components at 30 days post-hatching on chick growth and immune function at fledging, and b) long-term effects at sexual maturity (>90 days post-hatching) on phenotypic quality of i/ males (sons) using standardise mating trials (courtship, song rate, etc); ii/ females (daughters) by measuring reproductive traits during breeding (egg size, clutch size etc), and iii/ cell-mediated and humoral immunity in both sexes.

## A10.7 CHANGES IN WAVELENGTH OF LIGHT ALTERS GROWTH, GONADAL RESPONSE, HORMONES AND BLOOD PROFILE OF JAPANESE QUAIL, COTURNIX JAPONICA

WEDNESDAY 6 JULY, 2016 11:00

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Light colour or wavelength affects growth and reproduction reported in many avian species but reports are rare in Japanese quail. Further, blood parameters and hormonal changes are not well documented in quail. Three week male quail were exposed to LED light of different colours- White, Blue, Green and Red- having 30 lux of intensity. Control quail were kept in White fluorescent light (70 lux). All 5 groups were kept in LD 16:8 photoperiod and provided with food & water ad libitum. The Body weight and cloacal gland volume were recorded weekly upto 35 week of age. Statistical analysis showed that, after one week exposure, birds under all coloured LED light had higher body weight compared to that of white light. After two week exposure (5.5 week of age), green LED light had higher weight compared to other groups that was maintained upto 12.5 week of age except at 8.5 and 9.5 week age when it was lower than normal control although higher than other groups. After 12.5 week age, weight of green light quail was higher than other groups although it was not significantly different from red group. This condition was maintained upto 35 week of age. At this age, the corticosterone level, plasma testosterone concentration, H/L ratio, WBC, RBC, Hemoglobin, GSI and testicular volume were measured. The H/L ratio, WBC, RBC and hemoglobin were measured and were found to be altered in different groups. Our findings conclude that green and red light stimulate higher growth & reproduction without stress.

## A10.8 THE USE OF NANOTECHNOLOGY AND RNAI TO CONTROL AGRICULTURAL PESTS: CNT-DSRNA IN TRIBOLIUM BEETLES

WEDNESDAY 6 JULY, 2016 11:15

EWAN M CAMPBELL (UNIVERSITY OF ABERDEEN, UNITED KINGDOM), ANDREA CAPORALI (UNIVERSITY OF EDINBURGH, UNITED KINGDOM)

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Global food security depends on the effective control of agricultural pests. The current paradigm in pest control is increasingly unsustainable due to escalating resistance to multiple pesticide classes alongside uncertainty over collateral environmental harm. Two emerging technologies; Carbon nanotubes (CNTs) and RNA interference (RNAi), were combined to develop an innovative method to control the number one pest of stored food, and model organism, the Red flour beetle, *Tribolium castaneum*. Systemic and long-lasting depletion of target gene transcripts can be accomplished using dsRNA at any stage of *T. castaneum* development. The method of delivery of dsRNA, however, is critical in the silencing response. dsRNA can be micro-injected, orally fed and transgenically expressed in organisms, but silencing efficiency can be poor and remains a challenge to widespread adoption of this technology. We tested CNTs, functionalized with dsRNA, as a non-toxic drug delivery vector able to efficiently cross cellular membranes *in vivo*. CNTs, functionalised with dsRNA, to target the beetle gene transcript, *Atub* were injected into larvae. Relative *Atub*-mRNA levels were then quantified using qRT-PCR to estimate knockdown in beetle larvae. Significantly increased silencing of target mRNA and higher mortality was seen in CNT-dsRNA injected larvae compared with dsRNA-only treatments. Toxicity to CNTs was extremely low, as measured by dose response assays and qRT-PCR of apoptotic stress factors. TEM imaging indicated high levels of CNT-dsRNA complexes inside beetle cells. These results indicate that dsRNA-functionalised CNTs are amenable as low toxicity, high efficiency vectors to enable gene-silencing and mortality in agricultural pest species.

## A10.9 BIOPHYSICAL ANALYSIS OF NEURAL CHANGES UNDERLYING MEMORY IN AN INVERTEBRATE MODEL SYSTEM

WEDNESDAY 6 JULY, 2016 11:30

DANIEL PRICE (UNIVERSITY OF SUSSEX, UNITED KINGDOM), FELIX KERN (UNIVERSITY OF SUSSEX, UNITED KINGDOM), THOMAS NOWOTNY (UNIVERSITY OF SUSSEX, UNITED KINGDOM), ILDIKÓ KEMENES (UNIVERSITY OF SUSSEX, UNITED KINGDOM)

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The feeding system of the Pond Snail, *Lymnaea stagnalis*, is used as a simple model to understand the neural mechanisms of memory. Following a single pairing of a conditional stimulus, amyl acetate, and an unconditional stimulus, sucrose, an associative memory is formed that lasts for weeks. Underlying this is a persistent depolarisation of the cerebral giant cell (CGC), which plays an important role in the maintenance of long term memory. Further

analysis reveals no significant change in other cellular properties, such as firing frequency, action potential shape, excitability or membrane resistance. A computational model of the CGC has previously been created, based on Hodgkin-Huxley analysis of ionic conductances. This model predicts that an increase in three voltage-gated conductances, the persistent sodium current ( $I_{NaP}$ ), the delayed rectifier ( $I_K$ ) and the high voltage activated calcium current ( $I_{HVA}$ ), is sufficient to mimic the experimentally observed depolarisation. Our aim was to test this prediction using the dynamic clamp. During dual electrode recordings the three voltage-gated conductances taken from the model, were artificially added to the CGCs in non-trained snails, to simulate the effects of conditioning. This showed that, in their original form, the model conductances do not depolarise the CGC. Therefore modifications were made to the model of  $I_{NaP}$ , and the maximum conductances of the three model currents. Once this was done a depolarisation of the CGC could be achieved, without a significant change in spike frequency. This shows the importance of dynamic clamp in directly testing theoretical models on living neurons.

## A10.10 FEEDBACK INTEGRATION ON THE FLY – A MODEL FOR PHASE-CODED LOCOMOTOR CONTROL IN *DROSOPHILA* BY CYCLIC AND GRADED NEURAL INPUTS

WEDNESDAY 6 JULY, 2016 13:50

JAN BARTUSSEK (UNIVERSITY OF ROSTOCK, GERMANY), FRITZ-OLAF LEHMANN (UNIVERSITY OF ROSTOCK, GERMANY)

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Motor control in flying insects requires the integration of different sensory modalities such as feedback from eyes and mechanoreceptors. The impressive aerial performance of flies, in particular, depends on the temporal precise activation of wing steering muscles within narrow phase bands of the stroke cycle. Visual pathways from the compound eyes provide graded neural potentials during flight that are fused with phase-coded neural spikes from proprioceptive pathways. We studied this integration process on the level of motoneurons that drive wing steering muscles, using a numerical Hodgkin-Huxley model for neuronal function. All inputs were modelled as electrical synapses that transmit graded potentials from the visual interneurons and cyclic spike trains from the mechanoreceptors. We scored motoneuron firing frequency and the time relationship between wingbeat cycle and motoneuron spike initiation. Within a physiological range of parameters, our simulation shows that both cyclic and tonic feedback can modulate the timing of motoneuron spiking. These phase shifts alter the efficacy of muscle mechanical power output, in turn allowing modulation of wing kinematics and thus flight control. The model further implies that the impact of visual feedback on muscle activation phase strongly depends on the strength and temporal structure of the cyclic feedback. This agrees with experimental data and suggests a dynamic control of visuomotor gain by proprioceptive feedback. Collectively, our findings advance our understanding of rapid feedback integration during locomotion in insects by sensory feedback yielding different temporal structure.

### A10.11 LOCAL ANAESTHETIC? THE SYSTEMIC EFFECT OF SUBCUTANEOUS LIDOCAINE IN THE AMERICAN BULLFROG - *LITHOBATES CATEBEIANUS*

WEDNESDAY 6 JULY, 2016 14:05

CATHERINE J A WILLIAMS (AARHUS UNIVERSITY, DENMARK), AAGE K O ALSTRUP (AARHUS UNIVERSITY HOSPITAL, DENMARK), MADS F BERTELSEN (COPENHAGEN ZOO, DENMARK), CLEO A C LEITE (FEDERAL UNIVERSITY OF SÃO CARLOS, BRAZIL), TOBIAS WANG (AARHUS UNIVERSITY, DENMARK)

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Sodium channel blockers, such as lidocaine, are commonly used local anaesthetics; preventing processing of noxious stimuli (nociception), but their effects on the central nervous system and heart preclude their use at higher doses to induce general anaesthesia in mammals. Here we investigate the effects of subcutaneous injection of lidocaine (5 or 50 mg kg<sup>-1</sup>) in bullfrogs (*Lithobates catesbeianus*) on reflexes, gular respiration and heart rate (handled group, n=10) or blood pressure and heart rate via an arterial catheter (n=6). 5 mg kg<sup>-1</sup> lidocaine did not affect reflexes within an hour of injection, and caused no significant heart rate change in the handled group, but was associated with a reduction in gular respiratory rate (from 99 ± 7 to 77 ± 7 breaths min<sup>-1</sup>). The higher dose of lidocaine caused a further reduction in respiratory rate, no significant change in handled heart rate, but led to a progressive loss of righting reflex (complete loss by 50 min), palpebral reflex (n=8 loss at 70 min), and contralateral toe pinch withdrawal (complete loss by 70 min). Reflexes were regained over 4 h. Systemic anaesthetic effects were, however, not associated with anti-nociception, as a forceps pinch test at the site of injection provoked movement at the height of the systemic effect (70 min). Amphibians are routinely subject to general anaesthesia via exposure to sodium channel blockers such as MS222 or benzocaine, however caution should be exercised when using injectable lidocaine in amphibians, as it appears to dose dependently suppress reflexes, without necessarily preventing nociception.

### A10.12 THE EFFECT OF AMBIENT LIGHT ON VULNERABILITY TO PHOTODAMAGE IN CRUSTACEAN EYES

WEDNESDAY 6 JULY, 2016 14:20

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Light damage is a common challenge in animal eyes, and many animals adapted to living in very dim light conditions have eyes that are not only extremely light-sensitive but as a trade-off also highly vulnerable. A possible mechanism and the dynamics of photodamage were studied in a population of opossum shrimp *Mysis relicta*, which lives in a very dark Finnish lake (Pääjärvi) and has been shown to be both very sensitive to light and susceptible to light-induced damage. One remarkable feature of Crustacean

vision is the ability to remain functional over wide range of light intensities, which is at least partly due the bistable visual-pigment system, where metarhodopsin can be reconverted to rhodopsin by short-wavelength light. The mysids of our study population have a high retinoid content in their eyes, arguably to ensure efficient dark regeneration and a high concentration of native rhodopsin when the sparse and long-wavelength shifted illumination cannot support photoreconversion of metarhodopsin to rhodopsin. This might lead to excessive photon absorption under brighter light exposures, producing free radicals and causing protein and fatty acid oxidation. We thus hypothesized that the susceptibility to damage from stronger light exposures can be decreased by long-term acclimation to slowly increasing red background light, expected to drive the rhodopsin/metarhodopsin steady-state towards metarhodopsin. The results were broadly consistent with the hypothesis, although somewhat contradictory.

### A10.13 GETTING A LEG UP: USING ANIMAL-BORNE TECHNOLOGY TO MONITOR OLFACTORY COMMUNICATION IN CANIDS

WEDNESDAY 6 JULY, 2016 14:35

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Scent marking is an important, widespread, but poorly understood form of cheat-proof olfactory communication in mammals. Currently, the majority of the literature available on olfactory communication through scent marking is limited to captive animals or to wild individuals of relatively few species. This is partly because methods for studying wild animal scent marking are limited to manual tracking using conspicuous field signs, such as those left in snow. As a result, the research in this field is highly constrained; often confined to areas of sufficient snowfall, where conditions that do not typify the norm. New methods are urgently required so that researchers can put this important ecological phenomenon into proper context across a range of species and habitats. In this talk, we will report on efforts to develop a new methodology utilising animal-borne accelerometers to automatically monitor when canid species adopt a characteristic posture to scent mark. The method is illustrated on the easily trained and handled domestic dog (*Canis lupus familiaris*) but is applicable to all canids that adopt this posture. Thus, the method offers an exciting opportunity to study the olfactory communication of this diverse taxon, from ubiquitous pest species and urban colonisers to critically endangered canids.

### A10.14 INCREASED SENSITIVITY TO PREDATOR CHEMOSIGNAL L-FELININE IN MICE CORRELATED WITH ELEVATED FOS-IMMUNOREACTIVITY IN THE ACCESSORY OLFACTORY BULB

WEDNESDAY 6 JULY, 2016 14:50

VERA V VOZNESSENSKAYA (A.N. SEVERTZOV INSTITUTE OF ECOLOGY EVOLUTION, RUSSIA), TATIANA K LAKTIONOVA (A.N. SEVERTZOV INSTITUTE OF ECOLOGY EVOLUTION, RUSSIA), ILYA G KVASHA (A.N. SEVERTZOV INSTITUTE OF ECOLOGY EVOLUTION, RUSSIA), MARIA A KLYUCHNIKOVA (A.N. SEVERTZOV INSTITUTE OF ECOLOGY EVOLUTION, RUSSIA)

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Chemosignal (L-felinine) from domestic cat urine may be used by the house mouse to recognize potential predators, their physiological status and may affect hormonal status and reproductive output in mice (Voznessenskaya, 2014). Current study aims to examine whether early olfactory experience of mice with cat chemosignals may affect sensitivity to target odors later in adulthood and whether these changes in sensitivity correlated with neural activation in olfactory bulbs. We measured olfactory thresholds (OT) to cat urine/L-felinine using an automated olfactometer (Knosys, USA). Exposures of mice to cat odor (urine or L-felinine) during two weeks after eyes open, significantly lowered the OTs to cat urine ( $n=10$ ,  $p<0.05$ ) as well as to L-felinine ( $n=10$ ,  $p<0.01$ ) relative to controls. We performed immunohistochemical studies to identify neural substrate involved in reception and analysis of L-felinine. Mice were exposed intermittently (50% duty cycle) to 0.05% L-felinine ( $n=8$ ) or clean air ( $n=8$ ) for 45 minutes. Sections of olfactory bulbs were stained (c-Fos (4) sc-52, Santa Cruz Biotechnology; Alexa Fluor<sup>®</sup> 594, Life technologies). Sections were analyzed using Fluorescence Microscope (Keyence Bz-9000, Japan) with software. We recorded specific pattern of activation in accessory olfactory bulb (AOB). Neonatal exposures to L-felinine (0.05%) caused significant increase in number of Fos-positive cells in AOB in response to stimulation with L-felinine ( $n=8$ ,  $p<0.01$ ) as well we recorded an increase of activated area ( $n=8$ ,  $p<0.001$ ). Sensitization to L-felinine in mice correlated with elevated Fos-immunoreactivity in AOB in response to stimulation with the compound.

Supported RFBR grant 14-04-01150.

### A10.15 OLFACTORY SENSITIVITY OF THE SENEGALESE SOLE (*SOLEA SENEGALENSIS*) TO CONSPECIFIC URINE, AND ITS POTENTIAL ROLE IN CHEMICAL COMMUNICATION

WEDNESDAY 6 JULY, 2016 15:05

PETER C HUBBARD (CENTRO DE CIÊNCIAS DO MAR, PORTUGAL), ELVIRA FATSINI (IRTA, SPAIN), IGNACIO CARAZO (IRTA, SPAIN), FRANÇOIS CHAUVIGN (IRTA, SPAIN), MANUEL MANCHADO (IFAPA, SPAIN), JOAN CERDÀ (IRTA, SPAIN), NEIL J DUNCAN (IRTA, SPAIN)

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Chemical communication is much better understood in freshwater than marine fish. The Senegalese sole is a marine flat fish wherein one bottleneck in aquaculture is poor reproductive performance of captive-bred males; the industry relies on wild-caught broodstock. This study was undertaken to assess whether chemical communication plays a role in reproduction and, if so, whether problems in this system may contribute to the lack of reproductive success. Urine was collected from adult fish, wild and cultured, during the spawning season (March to May), and tested for olfactory potency using the electro-olfactogram (EOG). The effect of mature female urine on circulating luteinizing hormone (LH) levels was also tested in adult fish. Conspecific urine proved to be a potent olfactory stimulus for both immature and adult conspecifics, evoking large-amplitude, concentration-dependent EOG responses, with thresholds of detection around  $1:10^6$ . However, the form of the concentration-response curves depended on the sex and state of maturity of both the urine donor and the receiver. The majority of olfactory potency could be extracted by C18 solid-phase cartridges. Furthermore, the olfactory potency differed between wild-caught and captive-bred fish. Contrary to expectations, however, urine from wild-caught females was less potent than that from captive-bred females. Urine from mature females evoked a slight, but significant, increase in circulating LH levels 3 and 30 minutes after exposure. These results strongly suggest that urine-released odorants play a role in reproduction in the Senegalese sole, and that a fault in this system may contribute to poor reproductive success in captive-bred fish.

### A10.16 DIFFERENCES IN RENAL CAPACITY AND THE CONCENTRATION OF COMPATIBLE OSMOLITES ARE UNDERLYING THE INTERSPECIFIC VARIATION IN DROSOPHILACOLD TOLERANCE

WEDNESDAY 6 JULY, 2016 15:45

JOHANNES OVERGAARD (AARHUS UNIVERSITY, DENMARK), TRINE OLSSON (AARHUS UNIVERSITY, DENMARK), ANDERS MALMENDAL MALMENDAL (UNIVERSITY OF COPENHAGEN, DENMARK), HEATH A MACMILLAN (AARHUS UNIVERSITY, DENMARK)

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Many insects, including *Drosophila*, succumb to the physiological effects of chilling. At species-specific low temperatures these insects enter a comatose state and lose the ability to maintain extracellular ion and water homeostasis. Over time, transmembrane ion-gradients dissipate and membrane potentials depolarize leading to the accumulation of chill injuries. In a series of integrative and comparative studies on five species of the genus *Drosophila* we have demonstrated that the Malpighian tubules of chill susceptible species lose  $\text{Na}^+$  and  $\text{K}^+$  selectivity at low temperatures. This contributes to a loss of ion balance, most notably a deleterious increase in hemolymph  $[\text{K}^+]$ . By contrast, the tubules of chill tolerant *Drosophila* continue to secrete a high  $[\text{K}^+]$  and low  $[\text{Na}^+]$  primary urine in the cold allowing them to maintain ion gradients and thereby avoid cold injury. These chill tolerant species are also characterized by lower hemolymph  $[\text{Na}^+]$  (before any cold exposure) than their chill susceptible conspecifics. Lowered hemolymph  $[\text{Na}^+]$  limits passive drift of ions away from the hemolymph and is correlated with preservation of extracellular water and ion balance. In these species, we find that hemolymph  $\text{Na}^+$  is replaced by other 'cryoprotective' osmolytes that maintain osmolality. Together these data show that cold adaptation involves adaptations to prevent passive drift of ions during cold stress, but also adaptations that ensure balanced temperature effects on active transport at low temperatures. Together, these adaptations ensure chill tolerant insects preserve homeostasis and avoid cold stress injuries.

### A10.17 THE UNDERLYING MECHANISMS THAT POWER THE OSMOREGULATORY ACTIVE INTESTINE IN RAINBOW TROUT MIGRATING TO SEAWATER

WEDNESDAY 6 JULY, 2016 16:00

JEROEN BRIJS (UNIVERSITY OF GOTHENBURG, SWEDEN), ERIK SANDBLOM (UNIVERSITY OF GOTHENBURG, SWEDEN), ANDREAS EKSTRÖM (UNIVERSITY OF GOTHENBURG, SWEDEN), HENRIK SUNDH (UNIVERSITY OF GOTHENBURG, SWEDEN), CATHARINA OLSSON (UNIVERSITY OF GOTHENBURG, SWEDEN), MICHAEL AXELSSON (UNIVERSITY OF GOTHENBURG, SWEDEN), NICOLAS PICHAUD (UNIVERSITY OF MONCTON, CANADA)

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When osmoregulating in seawater, euryhaline teleosts such as rainbow trout (*Oncorhynchus mykiss*) undergo a range of potentially costly metabolic modifications to organs such as the gills, kidneys and intestine. Whilst previous studies have shown that hypo-osmoregulatory processes facilitating the branchial excretion of ions incur a metabolic cost, relatively little is known about the metabolic processes driving the hypo-osmoregulatory functions of the intestine. The intestine has the potential to incur a significant metabolic cost since some of the mechanisms responsible for the absorption of monovalent ions (critical for the absorption of water) require ATP (i.e.  $\text{Na}^+/\text{K}^+$ -ATPases). In this study we transferred rainbow trout to seawater and examined their osmotic status,  $\text{Na}^+/\text{K}^+$ -ATPase activity, as well as whole animal and intestinal mitochondrial oxygen consumption during a 35-day acclimation period. As expected, plasma osmolality and  $[\text{Na}^+]$  of rainbow trout significantly increased upon exposure to seawater. The return of osmotic homeostasis coincided with a substantial increase in intestinal  $\text{Na}^+/\text{K}^+$ -ATPase activity. However, elevated  $\text{Na}^+/\text{K}^+$ -ATPase activity was not correlated with standard metabolic rate, state II or state III intestinal mitochondrial respiration rates. So how is the ATP required for the ion pumps generated? Further examination revealed that in addition to an increased anaerobic production of ATP (i.e. increased lactate dehydrogenase activity), intestinal mitochondria also generate ATP more efficiently by modifying the contribution of particular complexes (i.e. increased complex I-dependent mitochondrial respiration) in the electron transport system. The underlying mechanisms triggering this switch are intriguing and will be discussed at the upcoming meeting.

### A10.18 TISSUE SPECIFIC PATTERNS OF ACID-BASE REGULATION DURING THE POST-FEEDING ALKALINE TIDE IN FISH

📅 WEDNESDAY 6 JULY, 2016 ⌚ 16:15

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Feeding causes an alkaline tide in gastric animals (a rise in blood pH and bicarbonate). This is caused by the equimolar export of bicarbonate into the blood from the gastric gland cells to balance their secretion of acid into the stomach. Rainbow trout (15°C) voluntary feeding on a 3% (of body mass) meal, experienced the largest alkaline tide found in animals; +0.35 pH units and doubling of plasma bicarbonate after 6 h, which took 2 days and 3 days, respectively, to return to pre-feeding levels. Given the ubiquity of feeding, this is probably the most common and long-lasting, but least studied, acid-base disturbance fish experience. We studied whether the intracellular acid-base responses followed a similar pattern in three tissues; red blood cells, liver and white muscle. As expected due to greater buffering inside cells, intracellular pH variations were smaller (<half) in all these tissues compared to blood, but were highly variable in their timing. Peaks of alkalosis after meal ingestion occurred at 2 hours in erythrocytes and liver, 6 h in blood (pH<sub>e</sub>), and 24 h in white muscle. Recovery of pH took 48 h in blood and muscle, but longer in erythrocytes (72 h). This suggests erythrocytes may actively prolong intracellular alkalosis to support blood oxygenation and the metabolic demands of digestion. Intriguingly, liver pH<sub>i</sub> recovered fastest (24 h), and actually became acidotic from 48-96 h. This may reflect temporal changes in hepatic-portal blood chemistry after feeding (initially base from the stomach, then later acid from the intestine).

### A10.19 USING PERFUSED GILLS OF *OCTOPUS VULGARIS* AND *SEPIOTEUTHIS LESSONIANA* TO STUDY DIFFERENT STRATEGIES OF AMMONIA REGULATION WITH DIFFERENT LIFESTYLES

📅 WEDNESDAY 6 JULY, 2016 ⌚ 16:30

👤 PO-HSUAN SUNG (DEPARTMENT OF LIFE SCIENCE NATIONAL TAIWAN UNIVERSITY, TAIWAN), MARIAN Y. HU (INSTITUTE OF PHYSIOLOGY UNIVERSITY OF KIEL, GERMANY), DIRK WEIHRACH (DEPARTMENT OF BIOLOGICAL SCIENCES UNIVERSITY OF MANITOBA, CANADA), MENG-WEI LIN (DEPARTMENT OF LIFE SCIENCE NATIONAL TAIWAN NORMAL UNIVERSITY, TAIWAN), JIUN-HONG CHEN (DEPARTMENT OF LIFE SCIENCE NATIONAL TAIWAN UNIVERSITY, TAIWAN), PUNG-PUNG HWANG (INSTITUTE OF CELLULAR AND ORGANISMIC BIOLOGY ACADEMIA SINICA, TAIWAN), YUNG-CHE TSENG (DEPARTMENT OF LIFE SCIENCE NATIONAL TAIWAN NORMAL UNIVERSITY, TAIWAN)

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In the present study, we applied an in vitro perfused technique to explore different ammonia regulation strategies in octopus (*O. vulgaris*) and squid (*S. lessoniana*) gills since they behave diverse lifestyles ecologically. Here we reported that NH<sub>4</sub><sup>+</sup> accumulation can be observed in *O. vulgaris* gills at their blood NH<sub>4</sub><sup>+</sup> level lower than 300 μM whereas NH<sub>4</sub><sup>+</sup> excretion occurred at blood NH<sub>4</sub><sup>+</sup> level exceeding 300 μM. To compare with *O. vulgaris* gills, *S. lessoniana* gills accumulated NH<sub>4</sub><sup>+</sup> at relative lower levels (< 100 μM) and excreted NH<sub>4</sub><sup>+</sup> at blood NH<sub>4</sub><sup>+</sup> level exceeding 100 μM. In addition, a lower pH (pH 7.2) perturbation was found to significantly increase NH<sub>4</sub><sup>+</sup> excretion in *O. vulgaris* gills, but not in *S. lessoniana* gills. Consequently, these results indicated that cephalopod gills are essential excretory organs mediating NH<sub>4</sub><sup>+</sup> homeostasis. And the NH<sub>4</sub><sup>+</sup> capacity in gills varies in different cephalopods, inferring that this physiological appearance may reflect their different locomotion and habitations. The present work also highlighted this perfusion method to better understand the physiological process in highly ammonotelic mollusks.

### A10.20 FACING SALINITY CHANGES IN THE INTERTIDAL ENVIRONMENT: A PLATYHELMINTH'S STRATEGY FOR PREPARING FOR OXIDATIVE STRESS

📅 WEDNESDAY 6 JULY, 2016 ⌚ 16:45

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Intertidal organisms must daily cope with drastic changes in their environmental conditions (temperature, oxygenation, salinity, radiation...). This is usually accompanied by increased formation in reactive oxygen (ROS) and nitrogen (RNS) species, which, if not controlled, leads to oxidative stress. Intertidal organisms are usually capable to counteract these deleterious effects of ROS

and RNS formation, through behavioral and/or physiological mechanisms (e.g. enhanced production of antioxidants). *Macrostomum lignano* is used to test this hypothesis when exposed to environmental salinity changes. Animals demonstrated to be comfortable in a wide range of salinities, ranging from freshwater to hypersaline seawater (>60 ppt). Energetically speaking, higher salinities were the most expensive conditions, since we detected an increase in mitochondrial density accompanied by increased respiration rates. However, such modifications come at the price of an enhanced superoxide anion production (DHE staining), which is likely associated with a high caspase 3 upregulation (detected by RTqPCR). However, animals are still able to live at high environmental salinity, likely through the upregulation of several mitochondrial antioxidants. However, animals at low salinities decrease their respiration rates, have reduced activity and enter metabolic depression, but, show an upregulation of their GST-pi. If animals at low salinity are indeed facing metabolic depression (and, thus, functional hypoxia), the return to seawater may result in an oxidative burst, as it happens in fasting/re-feeding, hibernation/arousal or ischemia/re-perfusion situations. This increase in GST-pi could be interpreted as a 'preparation for oxidative stress' a mechanism to fight the free radical production that occurs upon returning to seawater.

### A10.21 THE EFFECTS OF L-CARNITINE ON BLOOD AND TISSUE PARAMETERS OF MALE RATS FED WITH DIFFERENT LEVELS OF FISH OIL

WEDNESDAY 6 JULY, 2016 POSTER SESSION

HALIL YAVUZ (SCIENTIFIC RESEARCH INSTITUTE OF SELCUK UNIVERSITY, TURKEY), PROF. DR. FIRUZE KURTOGLU (UNIVERSITY OF SELCUK FACULTY OF VETERINARY MEDICINE DEPT OF BIOCHEMISTRY, TURKEY)

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In this study, effects of L-carnitine (300mg/kg/day) applied intraperitoneally to male rats fed ration containing several proportions of fish oils for 30 days on plasma l-carnitine, lipid hydroperoxide (LPO), triglyceride, cholesterol and fatty acid levels; body weight values; plasma and tissue (liver and muscle) antioxidant enzymes (SOD, CAT) and glutathione (GSH) were investigated. As animal material, 72 Sprague-Dawley male rats that have 5-6 months of age were used in the study. The research lasted for 60 days. Rats were divided into 6 groups in each be of 12 rats and were fed in standard rat cages ad libitum[s]. Six experimental groups were formed in the study as follows; 1-Control; 2-Fish oil (1%); 3-Fish oil (5%); 4-L-carnitine (300mg/kg/day), 5-L-carnitine (300mg/kg/day) plus fish oil (1%) 6-L-carnitine (300mg/kg/day) plus fish oil (5%). Plasma LPO levels showed meaningful declines in carnitine supplemented groups in certain periods compared to groups in 2 and 3 which fish oil supplemented. Activities of enzymes significantly ( $P < 0.001$ ) increased on carnitine supplemented groups especially the 4<sup>th</sup> and 5<sup>th</sup> groups. In conclusion, it was found that carnitine applied by 300mg/kg/day to rats statistically affected the blood and tissue parameters. It was also evaluated; extra-carnitine may decrease triglyceride levels and increase blood and tissue antioxidant, while lipid hydroperoxide levels can be display controlling effect by carnitine administration.

### A10.22 THE EFFECTS OF VARIOUS LEVELS OF BORON SUPPLEMENTATION ON SOME PLASMA MINERAL AND METABOLITES OF WETHERS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

VAROL KURTOGLU (UNIVERSITY OF SELCUK FACULTY OF VET. MED. DEPT OF ANIMAL NUTRITION AND NUTRITIONAL DISEASES, TURKEY), PROF. DR. FIRUZE KURTOGLU (UNIVERSITY OF SELCUK FACULTY OF VETERINARY MEDICINE DEPT OF BIOCHEMISTRY, TURKEY), DR. ESAT SAMI POLAT (UNIVERSITY OF SELCUK FACULTY OF VETERINARY MEDICINE DEPT OF ANIMAL NUTRITION AND NUTR. DISEASES, TURKEY), DR. EMEL GÜRBÜZ (UNIVERSITY OF SELCUK FACULTY OF VET. MED. DEPT OF ANIMAL NUTRITION AND NUTRITIONAL DISEASES, TURKEY)

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In this trial, the effects of various levels (0, 15, 30, 45 ppm) of boron (B) supplementation to the wethers diet on serum Ca, P, Mg, glucose, ALP, triglyceride, total cholesterol, blood urea nitrogen, albumin and total protein levels were investigated. A total of 32 merino male wethers 8 months age were used as animal materials. These animals were divided into 4 groups consisting 8 animals in each and fed in individual cage. To limit the location differences each wether placed in each group was distributed randomly among the different compartments of the cage system. Before the experiment, all animals were weighed and were grouped in equal body weight mean. This trial was made in University of Selcuk, Veterinary Faculty Experimental Farm. The experimental period was 56 days. Before experimental period performed 15 days as training period. As a boron source, sodium borate was added to the diets. Boron was not supplemented to the diets of control while trial groups included 15, 30 and 45 ppm B respectively. For plasma analysis, blood samples were taken from the all wethers in each group by venajugularis into heparinised tubes on the 1<sup>st</sup>, 28<sup>th</sup> and 56<sup>th</sup> experimental days and were centrifuged. Plasma Ca, Mg, P, glucose, ALP, triglyceride, total cholesterol, blood urea nitrogen, albumin and total protein were determined by UV spectrophotometer. Boron additions significantly affected the serum Ca and ALP values at different periods during the experiment. In conclusion, boron might have beneficial effects on some blood parameters of wethers.

## A10.23 MOUSE (BALB/C NU) BREAST TUMOR STRUCTURE AND Na<sup>+</sup>, K<sup>+</sup>-ATPASE IMMUNOLocalIZATION

WEDNESDAY 6 JULY, 2016 POSTER SESSION

SABER KHODABANDEH (TARBIAT MODARES UNIVERSITY, IRAN), AMENEH AHRARI (TMU, IRAN), HALEH AKHAVAN NIAKI (UNIVERSITY OF MEDICAL SCIENCES BABOL, IRAN)

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The 4T1 cell line is a laboratory model used in the tumors biology studies. This cell line is very tumorigenic with high capacity to metastasize in different organs. In order to investigate the structure of the tumor and localization of Na<sup>+</sup>, K<sup>+</sup>-ATPase enzyme, the mouse (Balb/c nu) breast tumors (created via 4T1 cells) were examined by histology and immunohistochemistry methods. For histological study, the sections (4µm) were stained with hematoxylin and eosin, special IgGα antibody and FITC were also used for immunohistochemistry study. Tumor structure showed visible abnormality in proliferation and high mitotic geneicity in epithelial cells. Immunohistochemistry analysis showed significant immunofluorescence in the tumor cells, which could be obvious sign of abundance of Na<sup>+</sup>, K<sup>+</sup>-ATPase enzymes (as a marker for sodium potassium pump). The current results showed that tumor cells were rich of sodium potassium pump in their plasma membrane. Previous studies suggested the high gene expression levels of Na<sup>+</sup>, K<sup>+</sup>-ATPase in human breast tumors, which is in accordance with results of current research. We concluded that immunohistochemical study of Na<sup>+</sup>, K<sup>+</sup>-ATPase in tumor cell lines could be as an index in tumor studies.

## A10.24 COMPARATIVE ANALYSIS OF BARRIER CHARACTERISTICS IN RETINAL AND CEREBRAL VESSELS OF ZEBRAFISH

WEDNESDAY 6 JULY, 2016 POSTER SESSION

JIN HYOUNG KIM (SEOUL NATIONAL UNIVERSITY HOSPITAL, KOREA (SOUTH)), JEONG HUN KIM (SEOUL NATIONAL UNIVERSITY, KOREA (SOUTH))

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The blood-neural barrier (BNB), including BBB and BRB, is essential for the physiological integrity of the CNS vessel, which is formed by the CNS capillary endothelial cells which is typically surrounded by glial cell end-foot processes. Zebrafish have emerged as an advantageous model for studying vascular development and characteristics. Here we investigated the barrier characteristics of the retinal and cerebral vessel using flil1-EGFP transgenic zebrafish. First, the retinal vessel formation was analyzed. By 7 dpf, the retinal vessel was formed between lens and retina, where intercellular junctional complexes were already present between endothelial cells. Interestingly, NG-2 expression, but not GFAP, was colocalized with EGFP-positive cells of the retinal vessel. Among endothelial tight junction proteins, claudin-5 was expressed on EGFP-positive cells of the retinal vessel, whereas occludin and ZO-1 were not observed on the vessel. Contrast to the retinal vessel, the cerebral vessels were composed of EGFP-positive cells surrounded by GFAP-positive cells as well as NG2-positive cells, where tight junction proteins of ZO-1, occludin, and claudin-5 were diffusely

expresses on EGFP-positive cells. In addition, the retinal vessel was so leaky that a mixture of fluorescein tracers (2,000-kDa FITC-dextran, 10-kDa rhodamine-dextran, and 350-Da DAPI) diffusely infiltrated into all retinal layers, whereas no leakage was observed in the cerebral vessels. Our results suggest that, unlike retinal vessels of higher vertebrates, the retinal vessel of zebrafish shows insufficient characteristics to meet a functional endothelium-based CNS barrier, whereas the cerebral vessel has typical characteristics of CNS barrier.

## A10.25 BROWN FAT IN NEONATAL MICE WOULD BE REDUCED BY INTRAVITREALLY INJECTED ANTI-VEGF ANTIBODY

WEDNESDAY 6 JULY, 2016 POSTER SESSION

JEONG HUN KIM (SEOUL NATIONAL UNIVERSITY, KOREA (SOUTH)), DONG HYUN JO (SEOUL NATIONAL UNIVERSITY, KOREA (SOUTH)), SUNG WOOK PARK (SEOUL NATIONAL UNIVERSITY, KOREA (SOUTH)), CHANG SIK CHO (SEOUL NATIONAL UNIVERSITY, KOREA (SOUTH)), MICHAEL B POWNER (UCL, UNITED KINGDOM), MARCUS FRUTTIGER (UCL, UNITED KINGDOM), JIN HYOUNG KIM (SEOUL NATIONAL UNIVERSITY HOSPITAL, KOREA (SOUTH))

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Anti-vascular endothelial growth factor (VEGF) agents are the mainstay treatment for various angiogenesis-related retinal diseases. Currently, bevacizumab, a recombinant humanized anti-VEGF antibody, is trailed in retinopathy of prematurity, a vasoproliferative retinal disorder in premature infants. However, the risks of systemic complications after intravitreal injection of anti-VEGF antibody in infants are not well understood. In this study, we show that intravitreally injected anti-VEGF antibody is transported into the systemic circulation into the periphery where it reduces brown fat in neonatal C57BL/6 mice. A considerable amount of anti-VEGF antibody was detected in serum after intravitreal injection. Furthermore, in interscapular brown adipose tissue, we found lipid droplet accumulation, decreased VEGF levels, loss of vascular network, and decreased expression of mitochondria-related genes, Ppargc1a and Ucp1, all of which are characteristics of 'whitening' of brown fat. With increasing age and body weight, brown fat restored its morphology and vascularity. Our results show that there is a transient, but significant impact of intravitreally administered anti-VEGF antibody on brown adipose tissue in neonatal mice.

## A10.26 NEPHRON STRUCTURE AND IMMUNOLocalIZATION OF Na<sup>+</sup>, K<sup>+</sup>-ATPASE IN THE KIDNEY OF MOUSE (NUDE BALB /C)

WEDNESDAY 6 JULY, 2016 POSTER SESSION

SABER KHODABANDEH (TARBIAT MODARES UNIVERSITY, IRAN), SOMAYEH RAZAVI (TARBIAT MODARES UNIVERSITY, IRAN), MOHSEN ASUREI (PASTEUR INSTITUTE OF IRAN, IRAN), RAMAZAN BEHZADI (PASTEUR INSTITUTE OF IRAN, IRAN), S KAVOUSIAN (PASTEUR INSTITUTE OF IRAN, IRAN)

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Nude mouse is used frequently as a model in the study of cancer biology. Due the importance of the relationship between sodium-potassium pump and cancer biology, the nephron structure and immunolocalization of Na<sup>+</sup>, K<sup>+</sup>-ATPase in the kidney were examined in Nude mouse using of histological and immunohistochemical methods. Histological observation showed that the kidney of the Nude mouse is composed of two parts: cortex and medulla, the cortex is the analogue of the human kidney cortex and the medulla is the analogue of the human kidney medulla. Immunohistochemistry photographs showed that the Na<sup>+</sup>, K<sup>+</sup>-ATPase fluorescent, as an indicator of the presence of sodium-potassium pump, was absent in the glomerol and in the descending loop of Henle, and a good fluorescent was observed in proximal tubule, the thick ascending loop of Henle, distal tubules and collecting ducts, respectively. Given the importance role of Na<sup>+</sup>, K<sup>+</sup>-ATPase in homeostasis, these parts are the most important sites that the enzyme can be considered for studies of cancer biology. Due the importance of the sodium-potassium pump in the treatment studies of cancer, using of immunohistochemical method for studding of change in the intensity of Na<sup>+</sup>, K<sup>+</sup>-ATPase for the medications and their side effects on function of the kidney seems appropriate.

## A10.27 UNRAVELLING MECHANOTRANSDUCTION IN THE LOCUST EAR

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Rhodopsin, the light-transducing protein that underpins vision, was discovered 65 years ago. Olfactory and gustatory transduction channels have now also been identified and the operation of the sensory neurons themselves largely understood. In contrast, the identification of mechanosensory ion channels that underpin the senses of touch, hearing and proprioception has proved more problematic. Mechanosensory neurons bear multiple transduction ion channels, whose expression is scarce, and function depends on many other proteins. Stretch-sensitive neurons of insects (so-called chordotonal organs), which form the most sensitive mechanical detectors in animals, have emerged as a useful tool to identify candidate mechanotransduction channels and understand cellular mechanotransduction in general. Despite such progress, it is not known how mechanotransduction operates in insect stretch-

sensitive neurons and the identity of the mechanotransduction channels is still unclear. We have developed whole-cell patch-clamp recordings in the Muller's organ of the locust ear. We can acoustically stimulate the ear and, in conjunction with voltage protocols and pharmacology, record the transduction current and the resulting dendritic and axonal action potentials. The elementary events of mechanotransduction are quantal bumps or depolarisations (first recorded by Hill, 1983, *J. Comp. Physiol.* 152: 475-482), similar to those in photoreceptors, which summate to produce a graded potential. This graded potential leads to voltage-activated dendritic spikes, which propagate through the soma to the axon spike initiation zone. We are building the first quantitative description of mechanotransduction in stretch-sensitive neurons of insects with the goal of identifying the mechanotransduction channels.

## A10.28 COMPARATIVE STUDY OF EXTRA-RENAL ORGANS IN CEPHALOPODS: NH<sub>4</sub><sup>+</sup> HOMEOSTASIS IN GILLS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

PO-HSUAN SUNG (DEPARTMENT OF LIFE SCIENCE NATIONAL TAIWAN UNIVERSITY, TAIWAN), MARIAN Y. HU (INSTITUTE OF PHYSIOLOGY UNIVERSITY OF KIEL, GERMANY), DIRK WEIHRACH (DEPARTMENT OF BIOLOGICAL SCIENCES UNIVERSITY OF MANITOBA, CANADA), MENG-WEI LIN (DEPARTMENT OF LIFE SCIENCE NATIONAL TAIWAN NORMAL UNIVERSITY, TAIWAN), JIUN-HONG CHEN (DEPARTMENT OF LIFE SCIENCE NATIONAL TAIWAN UNIVERSITY, TAIWAN), PUNG-PUNG HWANG (INSTITUTE OF CELLULAR AND ORGANISMIC BIOLOGY ACADEMIA SINICA, TAIWAN), YUNG-CHE TSENG (DEPARTMENT OF LIFE SCIENCE NATIONAL TAIWAN NORMAL UNIVERSITY, TAIWAN)

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Cephalopods are highly active mollusks excreting ammonia as the major nitrogenous waste. In addition, these animals have successfully evolved different lifestyles to accommodate their own specific ecological niches. Therefore in this study, we hypothesized that different cephalopods (*Octopus vulgaris*, *Sepia pharaonis* and *Sepioteuthis lessoniana*) with diverse locomotory capacities may possess respective strategies for metabolic ammonia excretion. Although our perfused works have already prove that their gills behave as an important extra-renal organ that are responsible for accumulating and excreting NH<sub>4</sub><sup>+</sup>; however, such bi-phasic ammonia regulation in *O. vulgaris* was significantly different from that in *S. pharaonis* and *S. lessoniana*. Our further perfusion experiments also found that an extracellular acidosis only significantly increase NH<sub>4</sub><sup>+</sup> excretion in *O. vulgaris*. The diverse ammonia regulation capacities in gills may reflect their locomotion appearances among different species. Besides we conducted an adenylyl cyclase inhibitor KH7 that apparently decreased apical NH<sub>4</sub><sup>+</sup> excretion in gills of *O. vulgaris* and *S. pharaonis* which inferred that a cAMP-dependent process involving in the NH<sub>4</sub><sup>+</sup> excretion pathway.

### A10.29 PRE-ACCLIMATION TO LOW AMMONIA IMPROVES AMMONIA HANDLING IN COMMON CARP WHEN EXPOSED SUBSEQUENTLY TO HIGH ENVIRONMENTAL AMMONIA

WEDNESDAY 6 JULY, 2016 POSTER SESSION

JYOTSNA SHRIVASTAVA (UNIVERSITY OF ANTWERP, BELGIUM), SURJYA DATTA (UNIVERSITY OF ANTWERP, BELGIUM), AMIT KUMAR SINHA (UNIVERSITY OF ANTWERP, BELGIUM), RONNY BLUST (UNIVERSITY OF ANTWERP, BELGIUM), GUDRUN DE BOECK (UNIVERSITY OF ANTWERP, BELGIUM)

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We tested the hypothesis whether acclimation with low concentration of ammonia can facilitate the fish to tolerate subsequent sub-lethal ammonia exposure by activating ammonia excretory pathways. Common carp were pre-exposed to 0.27 mM ammonia (~10% 96 h LC<sub>50</sub>) for 3, 7 and 14 days. Thereafter, each of these pre-exposed and parallel control (without pre-exposure) groups were exposed to 1.35 mM high environmental ammonia (HEA, ~50% 96 h LC<sub>50</sub>) for 12 h and 48 h. Results show that ammonia excretion rate ( $J_{amm}$ ) was strongly inhibited (or even reversed) in control group following HEA. On contrary, pre-acclimated fish (typically 3 and 7 days) were able to maintain  $J_{amm}$  at basal level. The efficient ammonia efflux in pre-acclimated fish was associated with the up-regulation of branchial mRNA expression of ammonia transporters and exchangers. Pre-acclimation stimulated the expression level of Rhcg-amRNA; significant up-regulation was recorded during HEA exposure in pre-acclimated group relative to the control-HEA exposed group. No positive effect of pre-acclimation was noted for Rhbg. Relative to control, the transcript level of Na<sup>+</sup>/H<sup>+</sup> exchangers was remarkably elevated in ammonia pre-acclimated fish and remained higher during the subsequent HEA exposure. Similar trend was noted for mRNA expression of Na<sup>+</sup>/K<sup>+</sup>-ATPase, however, expression level of H<sup>+</sup>-ATPase remained unchanged in all the experimental conditions. In conclusion, our study clearly demonstrates that although the pre-exposure to a low dosage of ammonia did not induce that many measurable effects as such, it improves the tolerance to subsequent high ammonia exposure through priming mechanisms in ammonia excretory transcriptional processes.

### A10.30 DISRUPTION OF ION BALANCE FOLLOWS DEATH IN COLD STRESSED TROPICAL SHRIMP (*MACROBRACHIUM ROSENBERGII*)

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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The tolerance to low temperature exposures is of critical ecological importance to ectotherms and it is therefore also of interest to understand the physiology determining their critical thermal tolerance. Here we evaluate two popular physiological models of low

temperature tolerance in arthropods using the tropical freshwater shrimp *Macrobrachium rosenbergii*. One proposed model for the thermal limits of aquatic arthropods is the oxygen- and capacity-limited thermal tolerance (OCLTT), where the thermal limit is set by a failure of oxygen transport or aerobic metabolism beyond a temperature threshold. We are currently testing this model by investigating whether hypothermia compromises oxygen availability (causing a decrease in blood oxygen content) and/or if aerobic metabolism is compromised at low temperature (causing a rise in anaerobic metabolites). An alternative physiological explanation for the lower thermal tolerance has recently been described for a number of insect species where cold exposure disrupts ion balance through a progressive rise in extracellular [K<sup>+</sup>] that causes onset of cold injury and death. However we found from muscle and hemolymph samples that a disruption of ion balance did only occur after the onset of cold injury indicating that failure to maintain ion balance is not the cause, but rather a consequence of death.

### A10.31 DELIMITATION OF THE TIME SINCE DEATH BY ANALYSIS OF POST MORTEM MUSCLE DEGRADATION

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Estimation of the time since death plays an indispensable role in the resolution of criminal cases. The awareness of the precise time of death gives many further explanations about the circumstances of death, validates a witness's statement, assesses alibis and thus narrows the field of suspects. There are numerous methods proposed for time of death estimation, but just a few of them achieved practical importance. The available methods are still very inaccurate, limited to short post mortem periods and are also highly dependent on several influencing factors (e.g. temperature, humidity, cause of death...). Therefore it is necessary to improve and expand the range of methods substantially. In this study we take advantage of the post mortem degradation process of human skeletal muscle and correlate specific appearing degradation products with certain post mortem time periods. For this purpose we used SDS-PAGE and Western blotting to determine the degradation process of selected proteins (troponin T, desmin, tropomyosin) in muscle samples of 40 forensic cases. Additionally, casein zymography was performed for analysis of calpain activity. We could demonstrate predictable characteristic alternations in the protein profiles until the first 10 days post mortem. Further, we also analyzed influencing factors (temperature, BMI, age) that are likely to affect the degradation process. The obtained results show clearly the potential of post mortem protein degradation for the estimation of the time since death.

## A10.32 ACTIVE HEARING IS NOT JUST FOR VERTEBRATES

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Many lineages of animals have developed hearing— which is defined as a behavioural response towards acoustic stimuli. That ears can be more than passive receptors of impending sound waves was first discovered in vertebrates. Since that time, active mechanisms— defined as energy consuming feedback that increases either specificity or perceived loudness of a signal— have been shown to be present in some arthropods as well. There are three different ways arthropods can perceive sound: substrate-borne vibrations best-known in spiders, tympanal hearing as exemplified in locusts, and antennal hearing known from various flies from *Drosophila* to different mosquitoes. Being small imposes severe constraints on performance in any type of sensor. Nonetheless acoustic sensors in nature, shaped by millions of years of evolution for their task, can be highly acute and sensitive in terms of signal-to-noise ratio, miniaturization and effectiveness. There is still some debate about which mechanism and model conveys the best explanation for the amplification. This research builds on previous work on the ears of both the mosquito and *Drosophila*, to characterize the function and parameters of another animal lineage with antennal hearing: the midge (*Chironomus plumosus*). These ears will be investigated through 3D-Laser vibrometry experiments characterising them in relation to other antennal hearing organs. Later work will investigate their ears with  $\mu$ CT based morphology and behavioural studies. From this a better understanding of how animals, and humans, detect sound, as well as inspiration that could lead to novel types of engineered acoustic sensors could be gained.

## A10.33 V-TYPE H<sup>+</sup>-ATPASE AND NA<sup>+</sup>/ K<sup>+</sup> ATPASE CONTRIBUTIONS TO K<sup>+</sup> AND H<sup>+</sup> TRANSPORT IN DROSOPHILA GUT EPITHELIA

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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K<sup>+</sup> and H<sup>+</sup> activity profiles along the caeca and midgut of third instar *Drosophila* larvae were characterized using the Scanning Ion-Selective Electrode Technique (SIET). The presence of V-type H<sup>+</sup>-ATPases and Na<sup>+</sup>/K<sup>+</sup>-ATPase was examined using immunohistochemistry and ATPase activity assays. The roles of transport ATPases in energizing ion transport across the larval gut were investigated using blockers like bafilomycin, a V-type H<sup>+</sup> ATPase blocker, and ouabain, a Na<sup>+</sup>/K<sup>+</sup>-ATPase blocker. Blockers were applied to the basal membrane, and ion fluxes across the gut were measured by SIET before and after application of the blockers. Addition of bafilomycin to the basal membrane led to a decrease in

proton absorption along the caeca and midgut except the large flat cell zone of the middle midgut (MMG (LFC)). Bafilomycin also led to decreased K<sup>+</sup> absorption across the caeca, the anterior midgut and copper cells of the middle midgut, suggesting proton-dependent transport of K<sup>+</sup>. Proton absorption was decreased by acetazolamide, indicating carbonic anhydrase activity in all regions except the anterior midgut (AMG) and MMG (LFC). Addition of ouabain led to the increase of K<sup>+</sup> absorption along the caeca, the AMG, and MMG (LFC), suggesting a role for the Na<sup>+</sup>/K<sup>+</sup>-ATPase in these regions. Immunohistochemical evidence and ATPase activity assays also show the presence of V-type H<sup>+</sup>-ATPases and Na<sup>+</sup>/K<sup>+</sup>-ATPase along the caeca and midgut.

## A10.34 OMEGA-3 POLYUNSATURATED FATTY ACIDS RESCUE THE ABNORMAL BEHAVIORS IN *FMR1* KNOCK-OUT ZEBRAFISH (*DENIO RERIO*)

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Fragile X syndrome (FXS) is a most generally hereditary form of human mental retardation. It frequently induced by triplet repeat expansion (CGG) mutation in fragile X mental retardation 1 (*fmr1*) gene promoter, and resulted in absence of the fragile X mental retardation protein (FMRP) expression. The common symptoms of fragile X patients include learning disabilities, inattention, hyperactivity, anxiety, autistic behaviors, social impairments, as well as other behavioral abnormalities. Our previous results demonstrated that the behavioral abnormalities in *fmr1* knock out zebrafish such as hyperactivity, abnormal anxiety level, avoidance learning impairment and autism-like behavior. Therefore, we evaluated the possible therapeutic effects of omega-3 polyunsaturated fatty acids (n-3 PUFAs) on behavioral abnormalities in *fmr1* KO zebrafish. It is well-known that DHA and EPA are essential nutrients which can reduce the mortality of premature born infants, and they have been proved to enhance mental function in both aging and Alzheimer patients. Recently, n-3 PUFAs supplementation was proved to rescue the behavioral abnormalities in *fmr1* KO mice. In our results indicated that a reduction in total PUFAs of the *fmr1* KO zebrafish body was found. After 4 weeks of n-3 PUFAs dietary treatment might partially rescue abnormal behaviors, such as elevated anxiety level and avoidance learning impairment. We suggested that the lack of PUFAs may lead to the abnormal behaviors in *fmr1* KO zebrafish, and then n-3 PUFAs supplementation is a potential therapy agent for FXS patients.

### A10.35 METABOLIC RESPONSE OF GREEN ABALONE JUVENILES (*HALIOTIS FULGENS*: GASTROPODA) UNDER ACUTE HYPOXIA AND HYPERCAPNIA

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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The capability to sustain metabolic performance and energy homeostasis is essential to tolerate adverse environmental conditions, especially in species with limited movement capacity, such as the abalone (*Haliotis fulgens*). In their natural environment, abalone regularly experience hypoxia, hypercapnia and warming events, which are expected to increase in frequency and intensity in the future. To investigate the effects of acute exposure to low oxygen and high CO<sub>2</sub> at various temperatures, we exposed abalone juveniles to a temperature ramp from 18°C to 32°C at daily increments of 3°C, under hypoxia (50% air saturation), and hypercapnia (~1,000 e atm pCO<sub>2</sub>), both individually and in combination. We measured the rate of oxygen consumption (MO<sub>2</sub>) and metabolic response of gill using <sup>1</sup>H NMR spectroscopy. The MO<sub>2</sub> increased with temperature under either hypoxia or hypercapnia and reached similar values at the warmest temperature; however, hypoxia elicited higher MO<sub>2</sub> at lower temperatures. Contrastingly, the combination of both drivers produced a decline in MO<sub>2</sub> at the warmest temperature. NMR revealed that hypoxia and hypercapnia individually induced an accumulation of free amino acids and anaerobic end products at the warmest temperatures, suggesting protein degradation to fuel metabolism and that the critical temperature was surpassed. Under combined hypoxia and hypercapnia, amino acids, osmolytes and anaerobic end products already increase at intermediate temperatures, but decrease at warmer temperatures, which corresponds to the drop observed in MO<sub>2</sub>. These results suggest that simultaneous presence of hypoxia and hypercapnia hamper the energy metabolism and osmotic regulation lowering the critical temperature compared to their individual effect.

### A10.36 EFFECT OF DIFFERENT EXPOSURE REGIMES OF L-FELININE ON ESTROUS CYCLES IN THE HOUSE MOUSE

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Long history of coexistence of domestic cat (*Felis catus*) and the house mouse (*Mus musculus*) led to development of mutual adaptations. L-felinine is a unique amino acid found in the urine of domestic cat and select members of Felidae family. Our previous research showed that L-felinine may play a role of chemical signal for the house mouse. In current study we examined influence of L-felinine on regulation of oestrous cycles in female mice. Fecal estradiol levels were determined using ELISA technique (Immunotech, Russia). We used four groups of mice (n=38) at age of three months and applied the same dose of L-felinine (0.05%; 50 &mu;g) in different regimes: (1) continuous action during 12 days; (2) application at regular intervals for two hours daily; (3) spontaneous exposures; (4) control (water). We collected fecal samples from each female at the same time each day. Estradiol baseline was calculated individually; concentrations above the baseline were considered as a beginning of luteal phase of oestrous cycle (De Bruin et al. 2014). The data obtained indicate that L-felinine may affect the length of oestrous cycle in mice. The number of ovulations in animals under continuous exposure to L-felinine significantly increased (p=0.00498, n=10). At the same time we observed decline in number of cycling females in group 2 (p=0.0233, n=9). For group 3 we observed only a tendency to decrease in number of cycling females (p=0.0578, n=9). Different modes of exposure to L-felinine produced different effect on oestrous cycles in mice.

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### A10.37 MUSCLE DEVELOPMENT AND GROWTH IN THE BURBOT (*LOTA LOTA*)

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Myogenesis, particularly cellular patterning of trunk muscle formation, and muscle growth in teleost fish is a topic of great interest for researchers studying vertebrate development. This work has been extremely fruitful in the model species zebrafish (*Danio rerio*), but also in species of economic interest, such as salmonid fish. By contrast, much less is known about muscle development in species in other teleost taxa. Thus, the aim of the present study is to outline the development and growth of trunk muscle in the burbot (*Lota*

*lota*) - the only gadiform freshwater fish. Immunolabelling for slow and fast myosins and for proliferating cells as well as histological staining methods are used to investigate the patterns of myotomal myogenesis in different developmental stages of burbot embryos and larvae. The obtained results contribute to a better understanding of the mechanisms behind developmental muscle patterning in fish in general and the evolution of different body plans in teleosts.

### A10.38 COLD ACCLIMATION IMPROVES SURVIVAL AFTER CHILL COMA THROUGH AUGMENTED ION REGULATIVE CAPABILITIES IN THE MIGRATORY LOCUST, *LOCUSTA MIGRATORIA*

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Most insects have the ability to acclimate to changes in temperature such that thermal performance and tolerance tracks seasonal or even diurnal temperature variation. This is also true for the migratory locust, *Locusta migratoria*, which markedly changes its thermal tolerance when acclimated to either high or low temperature. Recent studies have shown that insect cold tolerance is closely tied to the insect's capacity for preserving extracellular ion homeostasis during cold stress. It is, however, not known if and how thermal acclimation affects homeostatic capacity in locust following high or low temperature acclimation. In the present study we acclimated locusts to high (31°C) and low temperature (11°C) before exposing them to a coma-inducing cold exposure (0°C) for up to 48 hours. We find that cold acclimated locusts have a faster recovery after cold exposure and that they exhibit a higher survival than their warm acclimated conspecifics. Measurements of intra- and extracellular ion concentrations showed that particularly K<sup>+</sup> -balance is disturbed during cold exposure and we find that this disturbance is proportional to the thermal tolerance. Thus, cold acclimated locusts are characterised by a smaller disturbance of ion balance compared to warm acclimated locusts and as a consequence of their improved homeostatic capacity cold acclimated locusts are better able to maintain membrane potential during cold stress. Loss of membrane potential is known to cause chill injury and apoptosis and we are currently investigating how thermal acclimation affect the ability of locusts to avoid cellular apoptosis/necrosis.

### A10.39 DELAYED CHILL COMA RECOVERY IS ASSOCIATED WITH DISTURBANCE OF ION BALANCE IN *LEPIDOPTERA*

WEDNESDAY 6 JULY, 2016 POSTER SESSION

SIGNE O JENSEN (AARHUS UNIVERSITY, DENMARK), MADS K ANDERSEN (AARHUS UNIVERSITY, DENMARK), JOHANNES OVERGAARD (AARHUS UNIVERSITY, DENMARK)

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Exposure to low temperature induces a state of chill coma in insects and chronic cold stress is known to cause a massive disturbance of ion homeostasis. Following a cold stress the insects will potentially recover and the underlying mechanisms have been associated with the ability to reestablish ion and water homeostasis to regain membrane potential and neuromuscular function. In this respect the time required to recover from chill coma is a common measure of chill tolerance. Lepidopteran insects possess a markedly different hemolymph ion composition and rely on different mechanisms to maintain membrane potential and it is unknown if cold injury is also related to disturbances of ion homeostasis in these species. Here we report that delayed chill coma recovery is also associated with disturbances of ion homeostasis in three lepidopteran insect species *Manduca sexta* (larvae and adults), *Bombyx mori* (larvae) and *Heliconius cydno* (adults). We found CCRT to increase after prolonged cold stress in all species/life stages investigated and increased duration of CCRT was associated with increased hemolymph K<sup>+</sup> concentration in *B. mori* and depolarization of the equilibrium potentials of K<sup>+</sup> ( $E_K$ ) in both larvae and adults of *M. sexta* while a tendency of depolarization of  $E_K$  was also found in *H. cydno*. These results show that lepidopteran insects, like previously investigated insects, suffer from disturbances in ion homeostasis during chill coma and that these disturbances affect their ability to recover from chill coma.

### A10.40 PRESYNAPTIC SHAKING B ISOFORM EXPRESSION ALTERS SYNAPTIC COUPLING BETWEEN AUDITORY SENSORY NEURONS AND THE GIANT FIBER OF *DROSOPHILA MELANOGASTER*

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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In a previous study we showed that the synapse between auditory Johnston's Organ neurons (JONs) and the giant fiber (GF) is structurally mixed, being composed of Neurobiotin- (NB) permeable gap junctions and chemical synapses. However, it is the electrical component of the synapse that is the primary functional one, and we have used an RNAi knockdown approach, along with electrophysiology, to determine that the innexin (invertebrate gap junctional protein) Shaking B (ShakB) is the one that is required both pre- and post-synaptically for functional transmission at this synapse. In addition, anatomical studies showed that ShakB

knockdown prevented NB coupling between GF and JONs and removed the plaques of ShakB protein immunoreactivity that are present at the region of contact. Specific shakB RNAi lines that are predicted to target the ShakB(L), or ShakB(N), isoforms alone did not reduce the synaptic strength, implying that it is ShakB(N+16) that is required in the presynaptic neurons. Overexpression of ShakB(N+16) in JONs caused the formation of ectopic dye coupling, including the addition of LY coupling where there was none before. Conversely, expression of the 'wrong' isoform, ShakB(N), in the presynaptic neurons inhibited dye coupling. We are currently investigating the possibility that gap junction proteins may have an instructive role in synaptic target choice.

### A10.41 ACTIVATION OF ENDOGENOUS RETINOIC ACID SIGNALING IS ESSENTIAL FOR SURVIVAL OF RETINAL GANGLION CELLS AFTER OPTIC NERVE INJURY

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Retinoic acid (RA) is important during development, in neuronal plasticity, and also in peripheral nervous system regeneration. Here we use the frog visual system as a model to investigate the changes in RA signaling that take place after axonal injury to the central nervous system. Immunocytochemistry was used to localize different components of RA signaling within sections of the retina and optic tectum, namely, the enzyme retinaldehyde dehydrogenase (RALDH), and the retinoic acid receptors (RARs). All the components of RA signaling were present at low to moderate levels in retinas and tecta of control, unoperated animals. In retina, soon after optic nerve injury, there was a large increase in RALDH and also a large increase in retinal ganglion cell (RGC) RAR expression. We applied antagonists of RA signaling intraocularly and quantified the effects on RGC survival six weeks after axotomy, using a retrograde fluorescent tracer to label the neurons. Inhibition of endogenous RA signaling significantly decreased RGC survival, reducing it by about 50%. Inhibition of RA synthesis and RAR activity also abolished the axotomy-induced activation of the Erk signaling pathway. We conclude that the activation of RA signaling is an essential step in the survival of RGCs after axotomy. Further study of the mechanisms involved in this process will help to understand the potential therapeutic value of retinoic acid to treat nerve injury.

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### A10.42 ADAPTATION OF STANDARDIZED TEST TO ASSESS OLFACTORY FUNCTION

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Recently new arguments were raised against classic theory assigning humans to microsmatics, i.e. mammals with poorly developed olfaction and insignificant role of smell (Shepherd, 2004; Lundstrom, Olsson, 2010). Despite having less olfactory receptors than dogs and mice (Niimura, Nei, 2005), humans as any mammal respond to olfactory conspecific body odors. Comparative behavioral research carried out on different mammalian species and humans, showed that sensitivity to odors is not directly related to receptor pool size and neuroanatomical substrate (Laska et al, 2015). Currently, there are no standardized tests in Russia for quantitative assessment of human olfactory function, which slow down the development of the research in this area. Apart from the obvious practical value, the development of such a test will allow to compare the data for Russian population with others. In aim to adopt the University of Pennsylvania Smell Identification Test (UPSIT) for Russian population we tested more than 250 healthy subjects of different age living in megapolis and in the rural area. Average scores of correct answers out of 40 odor samples in group one (age 18-60) were  $34.73 \pm 2.29$  and  $33.08 \pm 3.12$  for Moscow respondents and rural area people accordingly; in group 2 (60 age and older) -  $28.22 \pm 3.35$  for Moscow inhabitants and  $27.36 \pm 3.70$  for people from rural region. Average UPSIT score for control group was  $33.08 \pm 2.87$ . The criterion for inclusion certain odorants into test is 75% of indentifiability. Based on the criteria we replaced odorants in the test and reviewed scoring instructions.

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### A10.43 EFFECT OF INCUBATION TEMPERATURE ON BODY GROWTH AND MUSCLE DEVELOPMENT IN TWO ECOTYPES OF WHITEFISH *COREGONUS LAVARETUS*

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Several lakes around the world are inhabited by ecotype pairs of teleost fish that differ in habitat utilisation, spawning behaviour and maximum size (dwarf type and regular type). Such ecotype pairs provide well-suited model systems for study of intraspecific phenotypical diversification. Here, we present data that demonstrate that thermal imprinting, the thermal experience in

embryonic life, aids the segregation of small and large forms within an ecotype pair of whitefish *Coregonus lavaretus*. Batches of fish of each form were kept at 2°C and 6°C 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°C-fish much larger than 6a-fish), indicating that in their case, normal spawning site temperature (6°C) is indeed likely to act as a growth limiting factor. In addition, immunolabelling was performed to quantitatively examine Pax7+ muscle precursor cells including such that are mitotically active (Pax7+/H3P+) or have entered differentiation (Pax7+/MEF2+). Results demonstrate that incubation temperature has an important influence on the proliferation/differentiation balance of such cells in the two ecotypes. This is of major significance to aspects of ecological developmental biology, fisheries biology and from the evolutionary perspective.

### A10.44 CASTING NETS - CATCHING NEUTROPHILS IN THE ACT

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Neutrophil extracellular traps (NETs) are meshworks of extracellular DNA decorated with microbicidal and cytotoxic proteins. NETs are formed by polymorphonuclear neutrophils during a special form of cell death termed NETosis. Evidence has accumulated that NETosis is a basal mechanism of vertebrate innate immune response which also contributes to inflammation and tissue damage in various diseases. NETs comprise complex three dimensional reticular structures of DNA filaments of varying thickness down to fully decondensated double helix strands, often forming a continuous meshwork of wide expanse. Neutrophils can cast large areas of NETs by crawling during NET release but the exact process by which numerous neutrophils interact to form extended layers of interwoven NETs is hardly understood. To further investigate this, we employed a combination of light and electron microscopic methods including immunolocalisation techniques to analyse in vitro NET formation behaviour of human neutrophils isolated from peripheral blood and of murine neutrophils from bone marrow. Our results suggest a model in which at the onset of NET shedding, NET strands are preferably adhered to NET strands already present, or to micro-obstacles of other kind. Adhered strands are then elongated by the cells' crawling away from their initial positions. Physical contact of elongated NET strands with cells in early stages of NET formation or with non-NET-forming cells is frequent. We propose that active mutual physical contact between

NET-forming cells serves to support the successive initiation of NET release and the generation of extended multidirectional NET deposits.

### A10.45 EXTRACORPOREAL SHOCK WAVE THERAPY ACCELERATES SKELETAL MUSCLE REGENERATION

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Mammalian skeletal muscle tissue exhibits a remarkable ability to adapt to physiological stressors such as exercise or muscle damage. Particularly in professional sports, the regenerative capacity of skeletal muscle tissue is a major issue, as muscle lesions are among the most common sports-related injuries in athletes and optimal treatment still remains obscure. To support regeneration and speed up recovery, extracorporeal shock wave therapy (ESWT) could be a promising approach as it gained increasing importance in tissue regeneration in various medical fields. It has been shown that ESWT up-regulates the expression of several growth factors, leading to proliferation and differentiation of various stem/progenitor cells and to increased blood supply. The present study demonstrates accelerated regeneration of acutely injured skeletal muscle tissue. Muscle regeneration was induced by a standardised cardiotoxin injury in rat hindlimbs, and regeneration processes were investigated and compared in ESWT treated and untreated animals. Muscle samples were analyzed by histomorphometry, immunohistochemistry and western blotting with regard to fibre size, nuclear content, recruitment of satellite cells and blood supply. Therapy resulted in significantly increased fibre size and myonuclear content as well as in significantly enhanced expression levels of pax7, myoD, myogenin and CD31, indicating accelerated proliferation and differentiation rates of satellite cells as well as increased blood supply following ESWT.

## A10.46 ALFAXALONE ANAESTHESIA IN THE BALL PYTHON (*PYTHON REGIUS*)

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Injectable anaesthesia is well-established in reptiles and facilitates the sedation of wild-captured animals, induction of surgical anaesthesia and sedation for handling of potentially dangerous, and in some cases venomous, animals. However, vascular access in snakes is challenging, making it preferable to use an induction agent that provides innocuous anaesthesia upon intramuscular administration. Thus, we investigated alfaxalone as an intramuscular induction agent for snakes. Alfaxalone is a synthetic neuroactive steroid that produces sedation and muscle relaxation and can be administered safely intravenously or intramuscularly. Innocuous anaesthetic induction, when it was delivered intramuscularly, has been reported in several reptile species, including turtles, tortoises and iguanas, however there is little evidence of the use of alfaxalone in snakes. Six ball pythons (*Python regius*) were each administered three doses (10, 20 and 30 mg/kg) and the quality of anaesthesia was assessed. The time to loss of righting reflex and muscle tone, alongside respiration rate and the ability to intubate with an endotracheal tube were recorded. Preliminary results indicated that over-stimulation by testing reflexes at short time intervals led to variable induction times and hypersensitivity in the snout, so muscle tone was assessed every two minutes and righting reflex every four minutes. We also assessed the potential for analgesia provided by the anaesthetic using mechanical stimulation with a pinch to the tail tip with forceps. Our aim was to determine an appropriate dose of alfaxalone to allow endotracheal intubation, which would facilitate maintenance of surgical anaesthesia with other agents, for example inhaled anaesthetics.

## A10.47 THE PHYSIOLOGICAL EFFECTS OF MORPHINE IN THE SOUTH AMERICAN RATTLESNAKE *CROTALUS DURISSUS*

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Although morphine represents the gold-standard for analgesia in mammals, an effective opioid remains to be demonstrated in snakes; no reliable anti-nociceptive effect was reported for thermal nociceptive stimulation in corn snakes, and morphine does not appear to exert significant analgesia upon subcutaneous capsaicin injections in ball pythons. Here we report the physiological effects of morphine in *Crotalus durissus* – the South American Rattlesnake. Arterial catheters were placed under isoflurane anaesthesia and local bupivacaine with either intramuscular morphine at 10 mg kg<sup>-1</sup> or saline. Catheters allowed determination of heart rate, mean arterial blood pressure and plasma corticosterone concentration. Morphine administration at induction caused a tendency towards tachycardia throughout surgery and recovery. Corticosterone concentration also tended to be higher in the morphine-treated snakes (morphine, 0 hr [corticosterone] 479 ± 187 ng ml<sup>-1</sup>, 48 hr [corticosterone] 410 ± 175 ng ml<sup>-1</sup>), while the control group showed the expected tendency for postoperative decrease in corticosterone concentrations (control, 0 hr [corticosterone] 410 ± 175 ng ml<sup>-1</sup>, 48 hr [corticosterone] 211 ± 121 ng ml<sup>-1</sup>). There was a significant tachycardia in snakes when morphine was administered post-operatively; with heart rates of 38 ± 11 beats min<sup>-1</sup> in morphine and 22 ± 7 beats min<sup>-1</sup> in control snakes at 7 hours after intramuscular administration. This corroborates previous findings in ball pythons. In conclusion, morphine at 10 mg kg<sup>-1</sup> did not reduce heart rate or plasma corticosterone in South American rattle snakes when administered pre-operatively, and was associated with a significant tachycardia when administered at rest.

## A10.48 MOLECULAR AND NEUROANATOMICAL CHARACTERIZATION OF VASOPRESSIN/OXYTOCIN-TYPE SIGNALLING IN AN ECHINODERM

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Vasopressin/Oxytocin (VP/OT)-type peptides are a bilaterian family of neuropeptides that exert effects via co-evolved G-protein coupled receptors. Studies on vertebrates and protostomian invertebrates have revealed roles for VP/OT signalling in osmoregulation, reproduction and social behaviour. However, little is known about VP/OT-type signalling in deuterostomian invertebrates that occupy an "intermediate" position in animal phylogeny.

We have identified a VP/OT-type neuropeptide (asterotocin) in the starfish *Asterias rubens* by cloning a cDNA encoding its precursor and detection of the mature neuropeptide in nerve extracts using LC-MS-MS. We have also identified an *A. rubens* VP/OT-type receptor that is activated by asterotocin when heterologously expressed in CHO cells.

Using mRNA in situ hybridization and immunocytochemistry (with novel antibodies), analysis of the expression of asterotocin and its receptor in *A. rubens* revealed expression in the ectoneural epithelial layer of the circumoral nerve ring and radial nerve cords, with stained processes in the underlying neuropile. Asterotocin-expressing cells were also observed in the tube feet, body wall and cardiac stomach, and immunostained processes are present in the basal nerve ring of the tube foot, the sub-epithelial nerve plexus of the body wall and the basal epithelial nerve plexus of the cardiac stomach.

Consistent with the expression of asterotocin and its receptor in the cardiac stomach, pharmacological studies reveal that asterotocin triggers cardiac stomach relaxation (in vitro) and eversion (in vivo) in starfish. Furthermore, our data indicate that asterotocin may exert these effects by triggering neural release of another signalling molecule, which then acts as a muscle relaxant.

## A10.49 INFLUENCING FACTORS ON POST MORTEM PROTEIN DEGRADATION

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Time since death estimation (TDE) is a crucial aspect in forensic routine work. Available methods, however, all exhibit several restrictions on behalf of applicability. We lately presented a promising new TDE approach on the basis of skeletal muscle protein degradation in an animal model, as well as in human tissue. Furthermore, this method was recently deployed to gain time since death data in actual forensic cases, in which all other methods failed. However, certain aspects such as environmental influencing factors remain to be determined to achieve a broad applicability of this approach in forensic routine work. The aim of the present work is to investigate the role of the most important influencing factor i.e. temperature. For this purpose we exposed sacrificed mice to varying environmental conditions, and dissected muscle tissue at specific points of time post mortem. Samples were then analysed on protein degradation by means of SDS-PAGE and Western Blotting and compared to each other. We discuss our results in context with data from actual forensic cases and further describe the usefulness of the application of accumulated degree days (ADD), as a combined measure of time and temperature in time since death estimation.

## A10.50 TRANSCRANIAL MOTOR CORTEX EVOKED POTENTIAL ATTENUATION IN THE WISTAR RAT

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Transcranial electrical stimulation of the motor cortex is a widely used technique to evoke potentials in the spinal cord, peripheral nerves and skeletal muscles. The technique is used to monitor spinal cord integrity during surgery that can potentially cause permanent disabling damage. Thus, reliable recording of stimulation responses is vitally important. Owing to the nature of recording nervous activity, where low signal amplitude can cause difficulties detecting activity, signal averaging is used to improve signal/noise ratios, i.e. repeating the sequence of stimulation and measurement a number of times and averaging the individual recordings. This approach relies on the assumption of near perfect signal reproducibility between recordings. We report, using transcranial stimulation of the motor cortex in the Wistar rat, that stimulation rate highly affects both signal amplitude and signal complexity. Faster stimulation rates drastically reduce the amplitude and complexity of resulting evoked potentials measured in the femoral nerve, whereas slower rates improve them. Prolonged fast stimulation can even lead to complete ablation of stimulation responses. This discovery could have ramifications for how evoked potential studies of higher brain centers and neural networks in general are carried out. We propose a hypothesis of adequate repolarization time after stimulation in order to maintain stimulus response fidelity. It seems plausible that this adequate repolarization time increases, as neural networks size and complexity increases, as is the case if one moves from rats to humans. Enhancing evoked motor cortex potentials' detectability could lower the risk of spinal cord damage during surgery.

## A10.51 PUPS WANT MORE MILK? SHAVING MAY HELP

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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We observed previously that lactating golden hamsters (*Mesocricetus auratus*) were significantly limited in their reproductive performance at elevated ambient temperatures of 30°C in comparison to 5°C. What measures can be undertaken to enable lactating females to get rid of excessively produced heat? Fur removal may be one easy and relatively non-invasive measure to manipulate thermoregulation so we set out to compare energy intake, milk production and juvenile growth in shaved and unshaved golden hamsters. By shaving, thermal conductance and thus heat loss is maximised and heat flow between females and their environment is manipulated while the thermal conditions of the pups remain stable. Experiments involving shaved females so far turned out to be inconclusive so new model systems and experiments identifying the importance of fur insulation on maternal peak energy budgets are needed. Also, we aim to understand why different strains of laboratory animals might be differentially constrained by heat. Throughout three weeks of lactation, we assessed time courses of body weights, subcutaneous body temperatures, energy intakes as well as litter sizes and litter weights of shaved hamster mothers and unshaved controls. First results reveal already that shaving of females led to an increase in pup growth: their young were 22.3% heavier than pups of unshaved mothers at the time of peak lactation and even 90.4% heavier at the time of weaning. Our results point to an efficient manipulation to boost milk production.

## A10.52 FISH USE PRESSURE TO SENSE THEIR ABSOLUTE DEPTH

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Fish move freely through the water and must navigate in three dimensions. A sense of depth would offer a substantial adaptive benefit to fish during three-dimensional navigation. Hydrostatic pressure provides a global cue that varies linearly with depth, so it should be straightforward, in principle, for an Actinopterygii fish to quantify its depth from a measurement of absolute pressure using its gas-filled swim-bladder. However, it has been assumed that Actinopterygii fish cannot sense absolute depth using pressure; the main dispute being that the volume of gas inside the swim-bladder is varied to regulate neutral buoyancy, so it cannot act as a long-term steady reference. Recent theoretical work proposes that absolute depth might be derived during vertical movement by combining a measurement of speed with a measurement of the fractional rate of change in swim-bladder volume. Here we provide the first empirical evidence that Actinopterygii fish can localise their absolute depth with remarkable precision relying exclusively on their sense of pressure. We found that the Mexican tetra, *Astyanax mexicanus*, accurately learnt the depth of a food reward using absolute pressure alone. Further, when we experimentally manipulated the surrounding pressure, fish shifted their search accordingly with high accuracy. Our results reveal a previously unidentified function of the swim-bladder in Actinopterygii fish and highlight new sensory information that fish are using during navigation.

## A10.53 ANOXIA-REOXYGENATION DISRUPTS FEAR-AVOIDANCE CONDITIONING IN GOLDFISH (*CARASSIUS AURATUS*)

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Although fish of the genus *Carassius* are capable of recovering from prolonged anoxia exposure and subsequent reoxygenation, they experience tissue damage, including in the brain. However, it remains unknown if the brain damage induced by anoxia-reoxygenation alters behaviour or disrupts mental acuity. We examined if anoxia-reoxygenation negatively affected the ability of 21°C-acclimated goldfish to learn and recall a classical fear-avoidance conditioned response. The latency (i.e., response time) to fear stimulus in pre-conditioned fish was decreased by 22% 1 h after a 6 h anoxia exposure, but then increased by 47% after 24 h of reoxygenation. By contrast, the fear stimulus avoidance success rate was not affected by anoxia-reoxygenation. Goldfish exposed to anoxia and then conditioned commencing at 24 h post-anoxia exposure exhibited a 70% lower fear stimulus avoidance rate at the onset of training compared to fish that were conditioned without prior anoxia exposure. Fish exposed to anoxia prior to training also required 1.8- to 3.4-times longer (15 days) to become conditioned compared to fish that had not been exposed to anoxia (4-8 days). Combined, our findings reveal that learning and recollection of a fear-avoidance conditioned response is disrupted by anoxia-reoxygenation in goldfish. Research is continuing to determine if the severity of the altered behaviour and mental acuity in response to anoxia-reoxygenation is correlated with the extent of brain damage experienced.

## A10.54 EVOLUTION IN PROGRESS: THE INTRIGUING CASE OF THE LAMBDA (TYPE III) INTERFERONS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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### Background

Gene duplication, an ubiquitous biological phenomenon, is arguably the major driver of evolution and contributor to genetic robustness and is epitomised by the recently identified type III (lambda) interferons (IFNLs), a group of small homologous cytokines encoded by a gene cluster located within a ~55kbp region on the long arm of chromosome 19. IFNLs are functionally similar to type I interferons, but their activities are restricted by receptor specificity to cells of epithelial origin. They have attracted considerable interest since several genome-wide association studies revealed that single nucleotide polymorphisms in their non-coding regions strongly influence both spontaneous and treatment-induced clearance of some viral infections as well as influencing severity of sterile inflammatory responses. IFNLs clearly play pivotal roles in inflammation and immunity, but many aspects of their regulation and function remain unexplored.

### Methods and Aims

Various freely accessible genomics and bioinformatics resources were used to investigate the phylogeny and structure of the IFNL locus and its products in an attempt to discover more about the evolution and biological effects of variation.

### Results and Conclusions

Early duplication of an ancestral type I interferon produced a primordial IFNL gene, further duplications of which generated IFNL2 and IFNL3. The phylogeny IFNL4 gene remains enigmatic. Small differences in IFNL gene and transcript structure predict differences in turnover rates. Overall, the results support the idea that IFNL genes are 'modern' genes that are still actively evolving in response to environmental pressure.

## A10.55 COMPUTING WITH NUCLEOTIDES: THE MITOCHONDRION AS AN EVOLVABLE, SELF-REGULATING POWER OSCILLATOR

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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### Introduction

Highly Organized Tolerance (HOT) is a conceptual framework for studying complex networks, essential characteristics of which are meta-stability, robustness and the ability to self-regulate. Such networks cope easily with environmental fluctuations unless they are very large and 'unexpected'. Engineered and evolved networks depend on appropriately coupled and regulated power supplies for optimal performance.

Mitochondria, the organelles that generate most cells' power by oxidative phosphorylation, provide a platform for integration of the signals that control vital reactions as well as the energy needed for their execution. In multicellular organisms, individual cells' power requirements vary enormously, depending on many factors that include developmental stage, nutrient availability and ionic microenvironment. Mitochondria produce the signalling molecule superoxide as a by-product of respiration, which is also required to generate UMP, the common pyrimidine precursor. Regulated nucleotide supply is essential for a plethora of functions including mitochondrial maintenance.

### Aim and Methods

Production of an electronic model of mitochondrial nucleotide metabolism using and design concepts from synthetic biology applied to bioinformatics data.

### Results and Conclusion

We designed a simplified electronic model consistent with available data to show that (1) except under 'unexpectedly' stressful conditions, the mitochondrial reserve capacity for nucleotide supply exceeds demand and (2) flux through the mitochondrial HOT network is normally repressed by negative feedback which if removed beyond a critical period will result in network failure. Gene duplication and alternative processing of gene products ensures that suitable protein components are available to maintain function under different 'expected' conditions.

## A10.56 SETTING OF A PROTOCOL FOR THE STORAGE OF SCALLOP (*PECTEN MAXIMUS*) SPERM AND LARVAE

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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The development of breeding programs for aquaculture and the preservation of biodiversity justify the creation of cryo-banks where samples of semen and embryos are preserved. Moreover, the development of genomics opens new opportunities to improve the understanding of the relationship between phenotype and genotype. This research requires biological resources and tools allowing gamete storage. A short-term storage protocol for scallop (*Pecten maximus*) sperm was designed, which permitted a mean sperm motility in excess of 20% after 64h storage. The effects of the container, the diluent, the dilution and the antibiotic on sperm motility were investigated. Results indicate variation of sperm storage capacity in relation with animals' origin (hatchery or wild) as well as high inter-individual variability. The first experiment on scallop larvae cryopreservation was carried out successfully. After 72h post-thawing, survival ranged from 65% to 95%. Structural integrity of numerous larvae was, however, impaired. After 10 days rearing survival rate of the cryopreserved larvae was less than 1%, while 47±8% in the control.

## A10.57 EFFECTS OF DIFFERENT SALINITIES ON THE OSMOREGULATORY CAPACITY OF MEDITERRANEAN STICKLEBACKS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Anthropogenic pressure and climate change put southern populations of three-spined sticklebacks (*Gasterosteus aculeatus* L.) at risk. This is especially relevant for the mesohaline and freshwater populations living along the northern Mediterranean coast with an anticipated conflict for habitat and resources between these populations.

To initiate this study, individuals from the Rhone delta (mesohaline population) were sampled and acclimated for at least two weeks in freshwater (FW; 5‰), brackish water (BW; 15‰), and seawater (SW; 30‰). To explore their hydromineral mechanisms, blood osmotic pressure and gill  $\text{Na}^+/\text{K}^+$ -ATPase (NKA) gene expression of the  $\alpha 1a$  and  $\alpha 1b$  isoforms were determined. Furthermore, the NKA protein expression in the gill ionocytes and the remodelling of these cells were investigated through NKA immunolabelling.

Blood osmolalities of FW-, BW- and SW-fish were significantly different. Branchial NKA  $\alpha 1a$  and  $\alpha 1b$  expressions were also different with less NKA  $\alpha 1b$  in FW than in SW. Ionocytes in FW-fish gills were located along the lamellae and at their base, whereas, in SW-fish, these cells are restricted to gill filaments. Ionocytes appeared elongated in FW-fish but possess a round shape in SW-fish. Finally, electron microscopy revealed three different types of apical structures for these ionocytes: honeycomb-like structure and dome shape in FW, or deeply encrypted in SW.

Therefore, ionocyte morphology and NKA expression are salinity-dependent. This remodelling must be directly linked to the physiological homeostatic status reached by the fish. It also highlights that this Mediterranean mesohaline stickleback populations can rapidly acclimate to different salinity conditions and can easily migrate to freshwater.