Trophic contamination by pyrolytic and petrogenic polycyclic aromatic hydrocarbons: Effects on metabolic and swimming performance in zebrafish: Effects on metabolic and swimming performance in zebrafish (Danio rerio)

Julie Lucas, Isabelle Percelay, Laura Lyphout, Xavier Cousin, Pierre Miramand, Christel Le François

A1 – GENERAL BIOMECHANICS

Organized by: Peter Aerts (University Of Antwerp)

A1.1
Seasonal plasticity in the prey-capture behaviour of the Alpine newt *Ichthyosaura alpestris* (Salamandridae)

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Evolutionary transitions between aquatic and terrestrial environments are significant steps in vertebrate evolution. These transitions require major changes in many biological functions, including food uptake and transport.
The Alpine newt, *Ichthyosaura alpestris* is known to show a 'multiphasic lifestyle' where the adult changes from a terrestrial to an aquatic life, and again to its terrestrial habitat every year due to its breeding activity. These seasonal transitions are associated with dramatic changes in morphology, physiology, and behaviour, and result in distinct aquatic and terrestrial morphotypes. We hypothesized that these shifts go along with changes in prey-capture mechanics to maintain a sufficiently high performance in both environments.
We analysed the prey capture kinematics in the four possible modes:
- aquatic strikes in the aquatic phase;
- terrestrial strikes in the terrestrial phase;
- aquatic strikes in the terrestrial phase; and
- terrestrial strikes in aquatic phase.

A multivariate comparison detected significant differences between the phase-specific feeding modes. In both the aquatic and the terrestrial phase, *I. alpestris* uses a suction-feeding mechanism for capturing prey in water. By contrast, *I. alpestris* uses a jaw-based grasping mechanism, similar to the aquatic modes, for terrestrial prey-capture in its aquatic phase but an elaborate lingual-based prehension mechanism to capture terrestrial prey in the terrestrial phase. We conclude that *I. alpestris* shows a so far unknown amount of behavioural plasticity in prey-capture behaviour that is tuned to seasonal demands of performance exemplifying functional mechanisms behind aquatic-terrestrial transitions in vertebrates.

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13:45 Thursday 4th July 2013

A1.2
The role of lateral mouth expansion during suction feeding in Largemouth Bass

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Mouth cavity expansion for suction feeding in fishes is driven by three musculoskeletal modules:
- the dorsal expansion module;
- the ventral expansion module; and
- the lateral expansion module.

All three modules must expand both fast and forcefully, requiring substantial muscle power. Dorsal and ventral expansion modules increase buccal cavity height through neurocranium elevation and hyoid depression, respectively, while lateral expansion increases the mediolateral width of the mouth cavity through suspensorium abduction.
Less is known about the lateral expansion module because its motions are difficult to visualize with external video.
To study lateral expansion kinematics in relation to total mouth volume change and power, we measured 3D skeletal kinematics, muscle shortening, and intraoral pressure during suction feeding in largemouth bass (*Micropterus salmoides*). Skeletal kinematics were measured with X-ray Reconstruction of Moving Morphology (XROMM), which combines biplanar X-ray video with bone models to generate 3D animations of skeletal motion. These XROMM animations were also used to calculate instantaneous mouth cavity volume. Muscle shortening was measured with fluoromicrometry, which uses biplanar X-ray video to record distances between intramuscular radio-opaque markers.
Lateral expansion contributes substantially to mouth volume increase through abduction of the suspensorium and hyoid bars, and includes lateral rotations of the upper and lower jaws as well. These lateral expansion kinematics appear to be linked to dorsal and ventral expansion, suggesting that muscle power may be shared across the modules. These results also emphasize the importance of lateral expansion for increasing mouth volume during suction feeding.

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14:00 Thursday 4th July 2013

A1.3
Feeding with a sticky tongue: Adhesion performance in the horned frog *Ceratophrys cranwelli*

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Frogs are well known to feed by rapidly firing their adhesive tongues towards elusive invertebrate and vertebrate prey species. The timeframe to establish a sufficient contact between the tongue and the prey surface lies in the range of only a few milliseconds. These extremely short time intervals for adhesion, as well as the ability to adhere to a variety of different surfaces, make frog tongues an exciting biological adhesive system. Here we measured the adhesive strength of tongues in the horned frog *Ceratophrys cranwelli* (Anura: Ceratophryidae) against a glass surface *in vivo*.
We found adhesive stress to be on average at 3 kPa, with peak values of 17 kPa. The adhesive forces are in the range of 1.2 times the body weight of the frogs and clearly outweigh the body weight of the captured prey animals. Adhesive strength is positively correlated with the pressure during the impact of the tongue. We further quantified the area on the glass surface, which was covered by mucus after the feeding event. We found the adhesive strength to be higher during feeding trials at which only a little amount of mucus was spread over the glass surface.
Thus, a thin layer of mucus is more beneficial for a successful feeding event. Further our findings suggest that besides the presence of mucus, the structural and physical properties of the tongue surface play a critical role in generation of a strong and reliable adhesive contact.

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14:15 Thursday 4th July 2013

A1.4
Modelling the functional trade-offs of durophagous teeth

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Durophagous teeth exhibit a diversity of morphologies. Two hypotheses could be used to explain this variety in form: tooth shape is selected to prevent teeth from breaking; or to most effectively break the prey item. In any durophagous species, we propose that both of these competing hypotheses will have some influence on tooth morphology. We tested these hypotheses using canonical models to isolate the effect of different aspects of tooth morphology. We generated three series that varied by:

- occlusal concavity;
- cusp height; and
- how much of the occlusal surface is covered by the base of the cusp.

We used FEA to test how these model morphologies responded to loading regimes, and determine which shapes were most likely to deform under loading. We found that occlusal concavity led to less deformation than convexity, and that cusp morphology affected the deformability of the models, with taller and narrower cusps deforming the most.

To test how well the different model morphologies would be able to break prey items, we measured the force needed to break a snail shell. To reduce variation due to prey we used a rapid prototyper to mass produce shells of identical size, shape, and material composition. The convex tooth models and those with taller or narrower cusps needed less force to break the snail shells.

Taken together, these studies indicate a trade-off in durophagous tooth morphology, since effective shapes tend to be the same model shapes that are most likely to deform under heavy loads.

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14:30 Thursday 4th July 2013

A1.5
Patterns of variation in EMG and 3D kinematics during feeding in primates

Jose Iriarte-Diaz (University of Chicago, United States) and Callum F Ross (University of Chicago, United States)

In recent years substantial advances have been made in our understanding of the feeding mechanics of primates. On one hand, researchers have investigated the relationship between the three-dimensional displacement of the mandible and food material properties as well as species-specific differences. Muscle activation patterns have also been recorded in multiple species and patterns of variation have been identified at different hierarchical levels suggesting the importance of intra and inter-individual variability. This variability derives in part from the structural complexity and redundancy of the masticatory muscles. However, little work has been done on evaluating how differences in patterns of muscle activation relate to differences in mandibular movement. Such interaction, although often ignored due to lack of data, is essential to understand how the masticatory apparatus in primates can adapt to changes in food material properties and how this affects feeding behaviour.

Using a large dataset of 3D mandible kinematics and muscle activation patterns recorded simultaneously, we investigate the relationship between all these factors in three species of non-human primates: macaques (*Macaca mulatta*), capuchin monkeys (*Cebus* sp.) and baboons (*Papio anubis*). Our data show that capuchin monkeys present a distinct pattern of muscle activation associated to differential use of working and balancing sides of the deep masseter muscle, not present in the other studied species. This suggests clade-specific differences between Old- and New-World monkeys in the way they modulate their feeding mechanics in response to changes in food material properties and feeding behaviour.

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14:45 Thursday 4th July 2013

A1.6
Biomechanics of bite force in stag beetle fights

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Male stag beetles use their large and elongated mandibles to fight each other for access over mates. To win, they have to grasp, lift and throw the opponent backwards over their own body. Unless this relies purely on geometrical interlocking, this fighting behaviour suggests the capacity to deliver high transversal bite forces, despite the long mandibles (i.e. output levers).

Using micro-CT scans of *Cyclommatus metallifer* stag beetles, we computed the theoretical difference in male and female bite force. Incorporating muscle cross-sectional area, muscle fibre direction and lever ratio of the mandibular system, this predicted – when normalized for size – a twofold bite force for males compared to females. *In vivo* measurements of maximal bite force performance, revealed a similar ratio (after size normalization).

These results demonstrate that the intersexual bite force difference can totally be attributed to morphological adaptations, whereas the force generating capacity (i.e. maximal muscle stress) remained unchanged. They also suggest that high bite forces are indeed a determining factor to win fights. So, sexual selection not only enlarged male stag beetle mandibles, but also drastically altered male head morphology to arrive at the observed bite forces. Therefore, the whole male head (and not only the mandibles) should be seen as male weaponry, evolved by sexual selection.

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15:00 Thursday 4th July 2013

A1.7
Both efficiency and work loop power output of *Xenopus laevis* muscle increase at higher temperature

Rob S James (Coventry University, United Kingdom), Jason A Tallis (Coventry University, United Kingdom) and Frank Seebacher (University of Sydney, Australia)

Supplying energy to muscles is essential to support the normal functioning of animals in their environment, with variation in metabolism being related to variation in performance in ecologically important behaviours. Many species live in environments that vary in temperature on a seasonal basis and respond to such variation by reversibly altering their metabolic and muscle phenotypes to compensate for the effects of such long term thermal changes. Many studies have demonstrated that acute rises in temperature cause increased muscle power output, however, there is no consensus in the literature regarding the effect of acute temperature change on muscle efficiency.[1] Our aim was to determine the effects of acute temperature change on the oxygen consumption, power output and efficiency of plantaris muscle isolated from two groups of *Xenopus laevis* frogs acclimated to warm (25°C) and cold (15°C) temperatures.

We found that muscle power output was significantly higher at 25°C than at 15°C (p<0.0001; n=9 from each acclimation treatment). However, maximum oxygen consumption during work loop performance of isolated muscle was significantly lower at 25°C than at 15°C (p<0.0001), consequently the amount of oxygen used per Joule of work output was significantly greater at 15°C than at 25°C (p<0.0001). There was no effect of acclimation treatment nor an interaction between acclimation treatment and test temperature in any of these measures.

In conclusion, muscle efficiency increased at the higher test temperature, but was not affected by acclimation.

1. RS James (2013) *Journal of Comparative Physiology B*. DOI 10.1007/s00360-013-0748-1

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15:40 Thursday 4th July 2013

A1.8
Coleoid cephalopods do not express tissue-specific muscle myosin heavy chain isoforms

William M Kier (University of North Carolina at Chapel Hill, United States) and Justin F Shaffer (University of North Carolina at Chapel Hill, United States)

The protein myosin is the principal contractile protein found in all muscle. It is widely accepted that animals modulate contractile velocity through the expression of tissue-specific myosin isoforms differing in amino acid sequence and ATPase activity. Recent studies, however, have shown that the squid, *Doryteuthis pealeii*, does not express muscle-specific myosin isoforms. Instead, modulation of contractile velocity occurs through changes in the dimensions of the sarcomeres and myofilaments. In this study, we investigated whether this alternative mechanism of tuning muscle contractile velocity is found more widely in cephalopods.
We analysed muscle myosin heavy chain transcript sequences and expression profiles from muscles of cuttlefish, *Sepia officinalis*, and octopus, *Octopus bimaculoides*, to determine if these species, each representing different cephalopod Orders, express tissue-specific myosin heavy chain isoforms. We identified three and six different myosin heavy chain isoforms in *S. officinalis* and *O. bimaculoides* muscular tissues, respectively. All isoforms were expressed in all muscles studied from each animal, suggesting that *S. officinalis* and *O. bimaculoides* do not express tissue-specific muscle myosin heavy chain isoforms. We also examined the sarcomeric ultrastructure in the transverse muscle fibres of the arms of *O. bimaculoides* and the arms and tentacles of *S. officinalis* using electron microscopy and found that *S. officinalis* have shorter thick filaments in the tentacle transverse muscle compared with the arms.
In view of these results, it appears likely that coleoid cephalopods in general use ultrastructural modifications rather than tissue-specific myosin isoforms to tune contractile velocities.

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15:55 Thursday 4th July 2013

A1.9
The effect of selective motor unit activation on muscle force–velocity properties: The size principle may not pose a mechanical paradox

Natalie C Holt (Concord Field Station, United States), James M Wakeling (Simon Fraser University, Canada) and Andrew A Biewener (Concord Field Station, United States)

In vertebrate skeletal muscle, motor units with varying mechanical and metabolic properties are selectively recruited to produce the range of muscle outputs required to meet the demands of locomotion. However, our knowledge of muscle function is largely derived from maximally activated muscle where all motor units are active. This discrepancy may limit our understanding of motor unit recruitment and *in vivo* muscle function more generally. In order to address this we determined the isotonic force-velocity relationships for rat plantaris muscle when all motor units were active and when either fast or slow motor units were selectively activated. There was a significant effect of active motor unit type on the relationship between whole muscle force production and shortening velocity (p<0.001). Maximum shortening velocity was lower when both slower (1.87 L_0 s^{-1}) and, surprisingly, faster (1.34 L_0 s^{-1}) motor units were selectively activated, than when all motor units were activated (3.52 L_0 s^{-1}). We attribute this to the increasing effect of the elastic, viscous and inertial properties of muscle that resist shortening with reduced activation level and suggest that there is an interactive effect between the proportion of the muscle active and the mechanical properties of active motor units on whole muscle mechanical performance. The findings presented here suggest that recruitment according to the size principle, where slow motor units are activated first and faster ones recruited as demand increases, may allow for the fastest shortening velocity and so may not present a mechanical paradox as has previously been suggested.

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16:10 Thursday 4th July 2013

A1.10
The mechanics of the steering muscles in blowflies revealed using time-resolved X-ray microtomography

Simon M Walker (University of Oxford, United Kingdom) and Graham K Taylor (University of Oxford, United Kingdom)

Flies are among the most agile of all insects and they achieve their fine control through an array of tiny steering muscles, which modify the much larger and symmetric output of the power muscles. Little is known about the mechanics of the steering muscles due to the difficulty in making measurements of fast-moving, micron-scale structures, which are also hidden from view by the thoracic shell.
Here we perform time-resolved microtomography of blowflies, *Calliphora vicina*, flying in a synchrotron beamline. This allowed us to visualize and measure the internal and external movements of the thorax through the wingbeat. The insects were rotated about their long body axis during the experiments, and produced compensatory roll responses involving asymmetric wing movements. We measured and compared the kinematics of five of the most prominent steering muscles between the high- and low-amplitude wings.
Our results show that all of these tiny muscles function as variable length springs, but that they control the wingbeat through a diverse range of nonlinear mechanisms. The muscles allow the flight motor to be switched between different modes of oscillation, and as well as modifying the resulting wing kinematics, they may also play an important role in power management during asymmetric manoeuvres.

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16:25 Thursday 4th July 2013

A1.11
Scaling of nerve fibre number in terrestrial mammals

Heather L More (Simon Fraser University, Canada) and Max Donelan (Simon Fraser University, Canada)

We investigated the relationship between the size of an animal and the number and size distribution of its nerve fibres. Physical limits to total nerve cross-sectional area create a competition between fibre diameter and fibre number, resulting in a trade-off between the speed and precision with which an animal can sense and respond to stimuli. This trade-off becomes more acute as animal size increases. A bimodal fibre size distribution would ameliorate, but not solve, the trade-off between fibre size and number, with large fibres allowing information to be conducted quickly and additional small fibres mitigating the decrease in precision. We therefore hypothesized that fibre size distribution becomes increasingly bimodal as animal size increases, while innervation density (fibre number per unit mass) decreases.
To test our hypotheses, we used histological techniques and scanning electron microscopy to measure fibres in the sciatic nerves of animals spanning six orders of magnitude in size from shrew (*Cryptotis parva*) to elephant (*Loxodonta africana*) and supplemented this with data from the literature. Our preliminary results support our hypotheses, showing an increase in the dichotomy of fibre size and a decrease in innervation density as animal size increases. These findings suggest that larger animals have two distinct populations of fibres – large fibres for fast responses, and small fibres to improve precision.
Our findings also suggest that large animals may not be able to sense and respond to stimuli as precisely as smaller animals, which may force them to rely more on predictive mechanisms to control their movement.

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16:40 Thursday 4th July 2013

A1.12
Fatigue of insect cuticle

Eoin E Parle (Trinity College Dublin, Ireland), Jan-Henning Dirks (Trinity College Dublin, Ireland) and David Taylor (Trinity College Dublin, Ireland)

Many parts of the insect exoskeleton experience repeated cyclic loading. Although the cuticle of insects and other arthropods is the second most common natural composite material in the world, so far nothing is known about its fatigue properties, despite the fact that fatigue undoubtedly limits the durability of body parts *in vivo*.
For the first time, we here present experimental fatigue data of insect cuticle. Using force-controlled cyclic loading, we determined the number of cycles to failure for hind legs (tibiae) and hind wings of the locust *Schistocerca gregaria*, as a function of the applied cyclic stress.
Our results show that, although both made from cuticle, these two body parts behaved very differently. Wing samples failed after 100,000 cycles when we applied 46% of the stress needed for instantaneous failure (the UTS). Legs, in contrast, were able to sustain a stress of 76% of UTS for the same number of cycles to failure. This can be explained by the difference in the composition and structure of the material and related to the well-known behaviour of engineering composites. Final failure of the tibiae occurred via one of two different failure modes – crack propagation in tension or buckling in compression – indicating that the tibia is evolutionary optimized to resist both failure modes equally.
These results are further discussed in relation to the evolution and normal use of these two body parts.

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16:55 Thursday 4th July 2013

A1.13
Spider silk under high-rate impact: Energy management and mechanical properties

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Spider dragline silks are well studied due to their exceptional toughness, which is unsurpassed by any other known material. These impressive mechanical properties have been shaped by evolution due to their natural function: dragline silks are used to absorb the energy of the falling spider as the 'safety' thread, and also to dissipate aerial prey energy as the radial threads in orb webs.
The mechanical response of silk, in theory, should change with the rate at which they are deformed, which has direct implications for spider web function under different speeds of prey impact. We present work here which quantifies the mechanical response of single fibres of spider silks across a range of deformation rates, from low-rate tensile testing through to high-rate ballistic impact. In the process we have gained insights into a wide range of silk properties including energy dissipation mechanisms using air drag.

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17:10 Thursday 4th July 2013

A1.14
Citius, *altius*, *fortius*: Jumping kinematics and kinetics in two distantly related teleosts

Benjamin M Perlman (Wake Forest University, United States), Sandy Kawano (Clemson University, United States), Richard W Blob (Clemson University, United States) and Miriam A Ashley-Ross (Wake Forest University, United States)

Many fish stranded on land will use axial movements to generate C-jumps in efforts to return to water. However, mangrove rivulus, *Kryptolebias marmoratus* (Cyprinodontiformes), generate coordinated jumps on land using a tail flip to locate new food resources, avoid predators, escape poor water conditions, or return to water. How do the mechanics of such directed jumps differ from those of typical stranded fishes? We quantified and compared the ground reaction forces (GRF) generated during directed jumps by rivulus with those produced by similarly-sized largemouth bass, *Micropterus salmoides* (Perciformes), performing typical jumps of stranded fishes.
Individual specimens were placed on a force platform that recorded the GRF in three dimensions. Forces were normalized to the body weight of each animal. High-speed video cameras recorded jump duration (from initial movement to launching off the force platform) and jump trajectory with respect to the ground.
Horizontal forces were greatest for rivulus with peak GRF occurring at ~75% through the jump. Bass had the greatest vertical GRF, occurring at ~60% of the jump. Trajectory of the bass C-jump was ~90° with respect to the ground compared to lower jump trajectories in rivulus, leading to greater horizontal displacement in rivulus. Bass had faster jump durations (~40 ms to reach maximum body curvature), whereas rivulus reached maximum body curvature at ~75 ms.
While the jumps of bass strongly resemble aquatic fast starts, differences in force production and motion trajectory in rivulus may indicate using different motor patterns to increase duration (and thus impulse) of the jump.

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10:30 Friday 5th July 2013

A1.15
Automated three-dimensional-tracking of fish fin and body kinematics

Cees J Voesenek (Wageningen University, Netherlands)

Biomechanical analyses of fish swimming require accurate knowledge of the fish's motion, since the time dependent force distribution on the fish can be derived from the three-dimensional motion of the skin through the water by the application of computational fluid dynamics.[1] Hence, quantitative analyses of the force production during swimming require kinematic data of the fish's body and fins with a high-enough spatial and temporal resolution.
We have developed a software package that automatically tracks the position, orientation and shape of a fish from synchronised high-speed videos from multiple view angles. The deformation of a three-dimensional body model of the fish is described with a set of parameters (e.g., position, orientation, body curvature, fin curvature, etc). We optimize these parameters for the synchronised sequence of video frames to achieve maximum overlap between the body model and video. The result is the complete three-dimensional motion of the fish, including its fins.
The software was applied to synchronised high-speed videos of zebrafish larvae from up to three different viewpoints. The resulting accurately quantified kinematics demonstrate that previous two-dimensional tracking approaches to fish larvae that use single (approximately) dorsal or ventral views fall short because fish larvae tend to make substantial pitch and roll movements and a single image is insufficient to reconstruct fin shapes.
1 Li G, et al. (2012). *J. Exp. Biol.* 215, 4015-4033.

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10:45 Friday 5th July 2013

A1.16
Body dynamics of larval fish – top performance by tiny athletes

Johan L Van Leeuwen (Wageningen University, Netherlands) and Ulrike K Mueller (California State University Fresno, United States)

Body and centre-of-mass (CoM) dynamics are fundamental to the mechanics of locomotion. Small swimmers and flyers are relatively strong, since they generate forces that are large compared with their body weight. However, small organisms must overcome relatively high drag forces, and have low efficiency. Here, we quantify the CoM kinematics of swimming zebrafish larvae from video recordings.
During cyclic swimming, the larval tail produces high torques as part of thrust generation: torque correlates with tail velocity (rather than tail acceleration). Torque increases with swimming speed, as does kinetic energy. A maximum power output, related to the observed changes in kinetic energy of 20 W/kg is observed at swimming speeds of 0.2 m/s at tail beat frequencies of 100 Hz. The actual power is higher due to the additional work done on the fluid. Peak powers approach the maximum power of superfast muscles. The Strouhal number decreases with increasing Reynolds number, from values above 2 at Re 100 to less than 0.8 above Re 1,000, indicating that efficiency increases with swimming speed.
Previous studies on C starts suggested that fish begin to translate in the preparatory phase (stage 1, formation of the 'C'). Our data show that the CoM translates very little but moves outside the body during stage 1. Translation rapidly increases during the propulsive phase (stage 2). Translational kinetic energy during stage 1 is near zero. Peak rotational kinetic energy is high during stages 1 and 2, reflecting substantial changes of heading generated by large torques.

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11:00 Friday 5th July 2013

A1.17
Function of fish schooling from the perspective of saving kinetic energy

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To examine whether the energy of fish swimming in schools can be saved more effectively than that of solitary fish, the swimming properties of fish were evaluated using tank experiments. Two different sizes of Japanese mackerel (*Scomber japonicas*) were used. In the constrained swimming test, four individuals were placed in a flume tank with changing current flow speeds. The tail beat frequencies of the followers were significantly lower than that of the leading individual when the flow speed was faster. The Strouhal number (St) of the follower in the school that was nearest to the leading fish, at a distance of <0.8 BL, was at a maximum 30% less than that of the leading fish.
Although the St of the leading fish was greater than the most efficient range of 0.2<St<0.4, that of the followers were in within this range. The oxygen consumption of the school was 30% less than that of which was excluded the hydrodynamic effects by schooling.
To evaluate the relationship between swimming kinetic energy and position within the school, we measured the three-dimensional swimming path of the free-ranging fish using a stereo camera system. We found that the tail beat frequencies of the followers were approximately 30% less than that of the leading fish when the flatness of the configuration of the school was greater. The tail beat frequencies of the followers within a range of 1 BL from the snout of the leading fish were 20% less than that of the leading fish.

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11:15 Friday 5th July 2013

A1.18
The propulsive role of pectoral fin movement changes with swimming speed: A computational study

Gen Li (Chiba University, Japan), Ulrike K. Müller (California State University Fresno, United States), Johan L Van Leeuwen (Wageningen University, Netherlands) and Hao Liu (Chiba University, Japan)

In order to explore the propulsive function of pectoral fins during larval fish's low-speed cyclic swimming, we have developed a three-dimensional, free-swimming and body-pectoral-fin multi-blocked fish model. Validation of the model was carefully done through the comparison of measured and computed body kinematics. Our computations revealed that the pectoral fins affect not only the propulsive performance of the larva, but also the flow pattern and force generation. The swimming mode combining the pectoral fin motions and the body undulations is more energy-consuming than body wave swimming with the fins against the body. Furthermore, an extended study on the effect of body undulation amplitude was conducted, demonstrating that the pectoral fin thrust is decreased while drag is increased with increasing undulation amplitude, which thrusts the fish to swim faster. It was also observed that the pectoral fin could solely provide forward propulsion when the body was kept straight without undulation. However, such propulsion effect turned to reduce and eventually became negative when the fish speeded up to some critical level. This explains why the larva is observed to have pectoral fins adducted during fast swimming. Our model predictions are supported by available measurements and reveal that the useful propulsive contribution of the pectoral fins is limited to relatively low speeds in larval swimming.

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11:30 Friday 5th July 2013

A1.19
Water temperature affects the swimming kinematics of rainbow trout, *Oncorhynchus mykiss*

Robert L Nudds (University of Manchester, United Kingdom), Emma Johns (University of Manchester, United Kingdom), Holly Shiels (University of Manchester, United Kingdom) and Adam N Keen (University of Manchester, United Kingdom)

An experiment whereby rainbow trout were swum at a range of steady state speeds (U) within a water flume at water temperatures of 11 (control) and 20°C was conducted. The latter temperature was selected because it is below the critical thermal maximum for rainbow trout, but above the hypothesized upper limit for successful migrations in salmonids. A temperature limit to migration success could indicate that swimming ability is impaired in some way. Here, the hypothesis tested was that increased temperature would induce a change in swimming kinematics (tail-beat frequency, *f* and amplitude, *A*) and/or energy expenditure.
Kinematics parameters were measured using high-speed video and the rate of O_2 consumption was measured using respirometry. Energy expenditure was elevated at the higher temperature, but the incremental increase with *U* remained similar. At 20°C the tail-beat kinematics were altered with a trade-off between *f* and A being apparent. The former increased and latter decreased at the higher temperature relative to the lower temperature.
The kinematics of oscillatory propulsion in animals seems to be configured to remain within a narrow range of Strouhal numbers ($St = f \bullet A / U$) associated with optimum propulsive efficiency. Temperature did not alter *St* here, suggesting that either *f* or *A* were influenced by temperature, possibly through an effect on muscle physiology, and the other parameter was then adjusted in order to adhere to an optimum *St*. It may be that swimming ability becomes impaired when scope for compensation in either *f* or *A* is exhausted.

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11:45 Friday 5th July 2013

A1.20
Wave-driven water motion affects escape responses in juvenile coral reef fishes

Dominique G Roche (Australian National University, Australia), Sandra A Binning (Australian National University, Australia) and Michael D Jennions (Australian National University, Australia)

Water motion is ubiquitous in aquatic systems but its effects on fish fast-start swimming performance are unknown. In shallow marine habitats, wave action creates unsteady water motion that can vary both spatially and temporally. Here, I examined whether wave-driven, unsteady water motion affects the escape response of juvenile coral reef fishes. I compared three species with contrasting body morphologies since deep bodies are thought to improve postural control and manoeuvrability compared to fusiform bodies. Escape responses were elicited under conditions of no flow or unsteady water flow. In the unsteady flow treatment, all fish covered a greater distance and achieved a higher speed and acceleration when escaping with rather than against water motion. Compared to the no flow treatment, however, unsteady flow had no effect on maximum escape performance, although fish took longer to respond to the stimulus. This latency effect depended on fish body shape. Fish with a more fusiform body responded slower in unsteady flow than in no flow, but the response time of fish with a deeper body was unaffected. This difference appears to result from fish with a deep body orienting themselves more often into the flow and being less displaced by water motion. Since response latency is a major determinant of escape success, postural disturbances from unsteady water motion might reduce the ability of some coral reef fishes to evade predators during settlement. This could have important implications for the distribution, abundance and recruitment of juvenile fishes across both spatial and temporal scales.

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12:00 Friday 5th July 2013

A1.21
Efficiency of pressure–volume work in swimming scallops

Richard L Marsh (Northeastern University, United States) and John M Olson (Villanova University, United States)

Scallops swim by jet propulsion with single muscle twitches occurring in each valve clap. Thus, this locomotor system does pressure–volume work in a manner resembling the vertebrate heart. All the work in scallop swimming is done by the striated adductor muscle. We measured the work done by swimming scallops using simultaneous measurements of pressure in the mantle cavity and calculations of the volume change between the rigid valves using sonomicrometry.
Following the swims the adductor muscle was removed and frozen for later measurements of the concentrations of ATP, arginine phosphate, and octopine. Measurements of metabolite values in the muscles of control scallops allowed the estimation of the total ATP consumed. A previous study in another species of scallop provides a value for the average free energy of ATP hydrolysis during swimming of 52 kJ/mol.[1] Based on this value our measurements indicate that the contractile efficiency (conversion of the free energy of ATP to work) in *Chlamys hastata* was approximately 30%. Values for *Argopecten irradians* have a larger uncertainty because of variability of the metabolite levels in control scallops. However, two separate trials found values of approximately 35 and 40%, suggesting strongly that the contractile efficiency is higher in this species.
Our data suggest that the efficiency of this pressure-volume system is at least as great as that found in vertebrate hearts (*Chlamys*) and may be greater (*Argopecten*).
1. Bailey et al. 2003. *Physiol. and Biochem. Zool.* 76, 622-633

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12:15 Friday 5th July 2013

A1.22
Geckos stabilize hard landings on a tree trunk after gliding assisted by active tails

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Here we show that integration of mechanics improves landing stability in an unspecialized gliding lizard. We measured geckos in a Southeast Asian rainforest to study tail function during aerial descent and gliding. Footage obtained in the field shows that *H. platyurus* is a capable glider, travelling significant horizontal distances in excess of 4 m. However, unlike specialized gliders, it does not undergo a stereotypical landing manoeuvre to substantially decrease velocity prior to impact.
Prior to landing geckos pitched their body to 27° and decelerated slightly to ~5 m/s. Hard collisions with the tree trunk pitched the torso vertically as high landing forces were absorbed by the body and tail. After vertical alignment with the tree trunk, geckos exhibited enormous pitch-back of their torso away from the tree to an average angle of up to 125° anchored by only the hind limbs and tail.
When geckos halted the backwards pitching at extreme peak pitch back angles of 103° 634° (n=3) toward the forest floor they maintained this extreme posture for an average duration of 19 msec ±. Of the gliding geckos that reached the tree target (n=7), the majority (86% of trials) alighted safely on the vertical target. By contrast, tailless geckos experienced catastrophic falls in 75% of trials after crashing into the tree (n=4).
Results reveal geckos use tails as shock-absorbers and stabilizers to reduce and control high impact forces acting on the limbs allowing effective landing at high speeds. Strategies incorporating tail-assisted control responses can improve the vertical landing performance and stability.

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13:15 Friday 5th July 2013

A1.23
Comparison between rigid and flexible primary feathers based on the wind tunnel experiments

Yoshinobu Inada (Tokai University, Japan)

Wing of birds is composed of lots of feathers. Among them, primary feathers play important roles in aerodynamic force generation. Previous studies clarified that the primary feathers can inhibit flow separation from a wing surface or attenuate induced drag by dispersing the wing tip vortices, etc.
Those findings were mostly taken from the experiments with rigid feather models and thus the function of flexibility in the primary feathers is not fully understood. We developed both rigid and flexible primary feather models and compared their aerodynamic characteristics by conducting wind tunnel experiments.
In the results, the flexible feathers did not show significant merits in the generation of lift or drag in a straight flight, but in a side slip flight, lateral force and rolling moment in the flexible feather model were significantly increased because the feathers deformed by the side wind increased the lateral projected area to receive more wind than the rigid non-deformed feather model. The increase of rolling moment can enhance the stability around the rolling axis which is called dihedral effect. The flexibility in the primary feathers, thus, can benefit the bird flight in the flight stability augmentation.

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14:30 Friday 5th July 2013

A1.24

Hovering aerodynamics of hummingbird with wing deformation: A computational study

Masateru Maeda (Chiba University, Japan), Toshiyuki Nakata (University of Oxford, United Kingdom), Ikuo Kitamura (Chiba University, Japan), Hiroto Tanaka (Chiba University, Japan) and Hao Liu (Chiba University, Japan)

Prior studies of flapping wing aerodynamics of insect flights with deforming and non-deforming wings have demonstrated the enhancement in aerodynamic performance owning to passive wing deformation. In this study, we aim at determining whether this mechanism still works in the smallest birds species by modelling deforming wings of a hummingbird based on measurements. Hovering flight of an amazilia hummingbird (*Amazilia amazilia*) was recorded with four synchronized high-speed video cameras at 2,000 frames per second in a greenhouse in Tama Zoological Park, Tokyo, Japan. We used the right wing images to reconstruct the wing kinematics and deformation; the left wing was assumed to have symmetrical shape about sagittal plane. With an unsharp mask, the wing edges and rachises (feather shafts) were tracked and extracted manually. The wing deformation was digitized and modelled in terms of the wing area, the camber, the span-wise bending and the span-wise twist in a complete wing beat cycle, which are thought to affect the flapping wing aerodynamics. The contribution to the aerodynamic force generation by each of these wing characteristics were then investigated using several types of wing models which are created based on the realistic (full fidelity) wing model. Our results showed that the significant variations in the wing area and the camber within the wing stroke exerted a direct impact on the aerodynamic force generation of the hovering hummingbird.

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13:45 Friday 5th July 2013

A1.25

Intraspecific variation in wing morphology and its impact on take-off performance within blue tits (*Cyanistes caeruleus*)

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Take-off is an important component of bird flight behaviour. It is an energetically demanding mode of flight as the centre of mass of the body must be accelerated, gain altitude and drag must be overcome on the wings and body. For maximal take-off performance the rate of gain in energy of the centre of mass should be high. Intraspecific variation in wing morphology (such as aspect ratio; AR, and wing loading; WL) result from moult, feather condition, sex, age and body mass, and likely impacts on take-off performance. The effects of intraspecific morphological variation on escape take-off performance were investigated in blue tits (*Cyanistes caeruleus*) via biomechanical and aerodynamic analysis. The rate of change of the energy imparted to the body's centre of mass was quantified to determine take-off performance, together with the total aerodynamic power requirements. We hypothesized that individuals with a high AR and a low WL would have increased take-off performance as these traits favour lift production and increased acceleration. It was found that individuals with lower WL had improved take-off performance. Individuals with a low WL have a relatively higher wing area favouring increased lift generation. However, because birds with a lower WL also had a lower AR, it was found, contrary to our hypothesis, that birds with a lower AR had increased take-off performance. These individuals exhibited higher wingbeat frequencies and relative down-stroke durations, factors that are likely to enhance flight muscle power output.

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14:00 Friday 5th July 2013

A1.26

The wing-tuck mechanism in a steppe eagle *Aquila nipalensis*

Kate V Reynolds (University of Oxford, United Kingdom), Graham K Taylor (University of Oxford, United Kingdom) and Adrian LR Thomas (University of Oxford, United Kingdom)

Turbulence is abundant in the surface boundary layer of the atmosphere, and birds are able to deal with, and potentially exploit, this to soar efficiently. Soaring birds can often be seen to pull their wings down, in a transient manoeuvre that we call a wing tuck. We analyse the mechanism, occurrence, and function of wing tucking in a trained, captive steppe eagle *Aquila nipalensis* using video and on-board inertial instrumentation.

Over 3,000 tucks were identified automatically from 60 flights. Statistical analysis revealed that the rate of wing tucking is positively related to atmospheric parameters which serve as proxies for mechanical turbulence intensity; specifically average flight altitude and local measures of the mean and fluctuating components of wind speed. The body motions preceding a tuck are reminiscent of the pitch-heave transient associated with vertical gust response in fixed-wing aircraft; load factor, pitch angle and airspeed all ramp up initially, but drop sharply before the wings are pulled down. The loss of load factor before the tuck is thought to be due to the abrupt change in angle of attack when the bird experiences a sudden gust but normal loading is quickly recovered once the wings re-open.

The results of an energy analysis are also presented here which further help to understand the function of the wing tuck as a gust response manoeuvre.

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14:15 Friday 5th July 2013

A1.27

Pigeon flock dynamics: Tight clusters *versus* loose formations

Steven J Portugal (Royal Veterinary College, United Kingdom), Alan Wilson (Royal Veterinary College, United Kingdom) and James Usherwood (Royal Veterinary College, United Kingdom)

When birds take to the air in a large group, they customarily fly in either a V-formation or a cluster-like swarm. Previously it has been demonstrated that there is a cost to flying in a cluster flock in pigeons, when circling around their home loft. This cost was in the form of a higher flap frequency and amplitude, assumed to be a result of aerodynamic interactions with other flock members, and the requirements for increased stability and control.

Using data from innovative custom-built GPS and accelerometer loggers, we will present research in pigeons which highlights the patterns in flap frequency and amplitude that are apparent when birds are flying over a distance of 12 km, from one location to another.

When flying in such a manner, there are typically larger distances between individuals within the flock in comparison to when birds are flocking as a cluster. Our data will investigate whether pigeons flying over long distances show traits more characteristic of larger birds that typically fly in V and J shape formations when travelling in a group over longer distances, as opposed to a cluster-like swarm.

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13:30 Friday 5th July 2013

A1.28

Aerodynamics and wing beat kinematics of escape manoeuvres in freely flying fruit flies

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Fruit flies and other insects possess a range of stereotypic flight responses that are triggered by particular sensory stimuli. Arguably, one of the most extreme flight responses is the evasive manoeuvre in response to looming stimuli, which enables a fly to avoid collisions as well as for escape from approaching predators.
Here, we study the aerodynamics and body and wing kinematics of visually-elicited evasive manoeuvres in freely flying fruit flies. Using an array of high-speed cameras (7,500 frames per second), we tracked body and wing kinematics throughout the behavioural response. The manoeuvres were triggered using a circular looming object displayed on the arena walls, which consisted of a cylindrical array of LEDs. The wing kinematics were extracted using an automated tracking routine and were replayed using a dynamically-scaled mechanical model of a fruit fly to study the aerodynamic forces and moments that govern the manoeuvre.
The free-flight experiments show that rapid evasive manoeuvres in fruit flies consist of four overlapping components: (1) a fast body roll, followed by (2) a counter-roll, (3) a pitch-up body rotation, and (4) an increase in total force production. As a result, the wing stroke plane, and as a consequence, the average flight force vector are directed away from the looming threat. Our results show that the fly controls roll, pitch, and flight force independently, by varying distinct kinematic features of its wing motion on a wing beat-to-wing beat basis.

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14:45 Friday 5th July 2013

A1.29

Altered wing shape improves flight performance in *Drosophila*

Richard J Bomphrey (University of Oxford, United Kingdom), Robert P Ray (University of Sussex, United Kingdom), Toshiyuki Nakata (University of Oxford, United Kingdom) and Per Henningsson (University of Oxford, United Kingdom)

The diversity of insect wing morphology seen in nature reflects the time-integrated sum of evolutionary pressures. Extant designs represent a compromise of ecological factors including – but not necessarily dominated by – aerodynamic performance characteristics. Correlating wing design with flight performance usually involves crossing species boundaries and can, therefore, be confounded by phylogenetic history.
Since biomechanical data sets can be technically challenging and time-consuming to obtain, implementing the comparative method is often unfeasible. One approach to this problem lies in the development of a standardized procedure for a single species that affords either discrete or continuous variation of morphological parameters that are expected, from aerodynamic theory, to play important roles in aerobatic capabilities.
Aerial prowess may be crucial to individual fitness and has certainly been instrumental in the success of the insects as a class. Thus, the ability to modify experimentally wing shape alone is a powerful tool with which to investigate the underlying mechanisms of functional morphology. Here we use RNA interference to knock-down expression of a gene that determines wing shape in fruit flies (*Drosophila melanogaster*). The resulting phenotypes differ markedly in shape but can be described by a single principal component warp. We used stereo photogrammetry to acquire three-dimensional free flight trajectories from the range of phenotypes, calculated manoeuvrability and agility metrics, and found them to be significantly enhanced by the modified wing morphology.

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15:00 Friday 5th July 2013

A1.30

Flight physics and visual physiology explain the function and mechanism of optomotor control responses of hawkmoths

Shane P Windsor (University of Oxford, United Kingdom) and Graham K Taylor (University of Oxford, United Kingdom)

The physiological flight control system of a flying insect works in concert with the insect's fight dynamics to precisely control the insect's motion through the air. In order to achieve this, sensory stimuli must be processed so as to extract the relevant information to allow the action of the motor system to stabilize and control the insect's flight dynamics. We quantified the flight control responses of the hawkmoth H*yles lineata* using a virtual reality flight simulator to measure the flight forces and head motions generated by the moths in response to visual motion. Modelling of the moths' flight dynamics showed that the measured responses were suitable for stabilizing instabilities predicted in their flight. We then computationally modelled the mechanism by which the moths process visual motion information using a model based on a moving array of Reichardt correlation detectors.
The output from this model corresponded closely with the insects' measured flight control responses. The model also showed that gaze stabilizing head motions act to greatly extend the working range of Reichardt detectors, and that the nonlinear response of these detectors can explain the non-linearities measured in the moths' flight responses. Taken together these results explain the function and mechanism of the optomotor control responses of hawkmoths.

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15:40 Friday 5th July 2013

A1.31

The effect of aspect ratio on the structure of leading-edge vortices over insect-like wings

Nathan Phillips (University of Oxford, United Kingdom), Kevin Knowles (Cranfield University, United Kingdom) and Richard Bomphrey (University of Oxford, United Kingdom)

Wing shapes exhibited by flying insects vary widely in aspect ratio. Computational studies have shown that the aspect ratio of an insect wing is an important parameter in determining the structure of the leading-edge vortex (LEV), a lift-augmenting flow structure exploited by many insects, bats, and birds. We conducted an investigation into the effects of wing aspect ratio on the formation and structure of the LEV in high spatial detail.
Experiments were accomplished with a custom-designed, mechanical flapping apparatus (the 'Flapperatus') that mimics insect-like kinematics, with adjustable wing beat frequency up to 20 Hz in air. This approach enables highly repeatable test cases, thereby allowing the effects on flow structures caused by changes in wing aspect ratio to be observed.
We made stereoscopic particle image velocimetry (stereo-PIV) measurements of the flow fields generated by rectangular wings with a range of aspect ratios. We used the data to characterize the formation and structure of the LEV, as well as skin friction lines over the wing surface that reveal the flow separation and reattachment lines that mark the 'footprint' of the LEV on the suction surface.

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15:55 Friday 5th July 2013

A1.32
Biomechanics of bamboo: What do the nodes do?

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Bamboo consists of a hollow, tubular culm with periodic nodes. These nodes clearly have a biological function, but do they also have a mechanical function? Previous researchers have claimed that the nodes, constructed with an internal diaphragm and external ridge, prevent failure by making the tube stiffer and stronger. We carried out tensile and bending tests on culms with and without nodes, and crack propagation tests to determine the effect of nodes on toughness.
Our results suggest that far from being a point of strength, the node is a point of potential weakness owing to the local branching and angulation of fibres. Material in the node is weaker and a potential source of cracks which spread along the culm. Nodes help to limit crack propagation but are too widely spaced to improve strength in the culm as a whole. Thus the diaphragm and ridge can be explained as an attempt to reinforce a biologically essential feature which would otherwise be a point of weakness.

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16:10 Friday 5th July 2013

A1.33
Slippery when wet: Functional morphology of the pitcher plant trapping zone

David Labonte (University of Cambridge, United Kingdom), Adam P Robinson (University of Cambridge, United Kingdom) and Walter Federle (University of Cambridge, United Kingdom)

Nepenthes pitcher plants capture prey with a specialized surface at the pitcher rim, the peristome, which becomes slippery only when wet. The peristome's function appears to be aided by radial ridge structures that have two different length scales: first-order ridges on the 100 µm length scale, superimposed on which are several second-order ridges on the 10 µm length scale.
In order to clarify the function of the first- and second-order ridges, we measured single-pad friction forces of *Carausius morosus* stick insects on dry and wetted peristome replicas and artificial surfaces patterned with ridges comparable in length scale to both first- and second-order ridges. Replicas were produced in transparent epoxy using silicone moulds of *Nepenthes maxima* peristomes; artificial ridge structures were made from the same material after taking moulds of ridge substrates fabricated by photolithography. Friction forces were significantly reduced on wetted substrates in all cases, but much more strongly on the surfaces containing second-order ridges. Dynamic contact angle measurements on fresh pitcher peristomes revealed that water rapidly spread and wetted the peristome along the ridges, but fluid movement was strongly limited across the first-order ridges.
Our results indicate that the second-order ridges alone are sufficient to stabilize the water films on which insects aquaplane, whereas the first-order ridges may help to confine wetting to channels running into the pitcher, thereby facilitating prey capture when only small amounts of water are present.

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16:25 Friday 5th July 2013

A1.34
Where adhesion fails: Slippery surfaces of insect-trapping pitcher plants

Ulrike Bauer (University of Cambridge, United Kingdom)

Pitcher plants (genera *Nepenthes*, *Heliamphora*, *Sarracenia* and *Cephalotus*) rely on specialized slippery surfaces to trap mainly insect prey to supplement their nutrition and enable them to survive on extremely poor soils. Early research in the first half of the 20th century found the slipperiness based on epicuticular layers of easily detachable wax platelets that reduce the available contact area and contaminate insects' adhesive pads. The recent discovery of a completely different, wetness-based anti-adhesive mechanism in *Nepenthes pitchers* suggested that trapping mechanisms might be more diverse that previously assumed.
Here I present two newly-discovered trapping mechanisms of pitcher plants. First, I demonstrate a wetness-enhanced trapping mechanism for *Heliamphora nutans*, a South-American species that is completely unrelated to the Asian *Nepenthes*. This does not only represent a striking case of functional convergence but also involves complete wetting of a hairy plant surface while similarly dense arrays of plant hairs (trichomes) are usually water-repellent. Second, I show that the lid of *Nepenthes gracilis* pitchers functions as an inverted springboard, utilizing the impact of rain drops to catapult insects into the trap. The stiffness of the lid material helps to transmit the impact, and unusual pillar-shaped wax crystals render the underside of the pitcher lid just slippery enough for insects to become dislodged.
These discoveries add to the striking diversity of trapping mechanisms in pitcher plants and highlight the importance of this group for the study of natural anti-adhesive surfaces.

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16:40 Friday 5th July 2013

A1.35
Functional morphology and efficiency of the antenna cleaner in *Camponotus rufifemur* ants

Alexander Hackmann (University of Cambridge, United Kingdom) and Walter Federle (University of Cambridge, United Kingdom)

In the course of evolution insects have developed a variety of strategies to reduce the contamination of their body surface, which can inhibit physiological functions. Many insects regularly pull their antennae through specialized cleaning devices on their forelegs. In ants, the antenna cleaner consists of a round notch on the basitarsus facing a movable spur on the tibia. Each component bears regularly spaced cuticular 'combs' and brush-like arrays of flexible hairs. Video recordings of the cleaning behaviour in *Camponotus rufifemur* ants showed that the spur helps to hold the antenna inside the tarsal notch. In order to investigate the function of spur and notch, we simulated artificial cleaning movements by moving individual cleaning structures over severed antennae that had been contaminated with fluorescent particles. Measurements of particle density on the antenna before and after simulated cleaning movements showed that the tarsal notch removed particles more efficiently than the tibial spur, but both components removed more than 60% of the particles with the first stroke. Removal of brushes and combs strongly reduced cleaning efficiency for both notch and spur. We contaminated the antennae of live ants with polystyrene particles of defined sizes and anaesthetized the animals immediately after a single cleaning stroke. SEM images revealed that different-sized particles became trapped in distinct zones according to the spacing between the cuticular outgrowths, suggesting that these structures are sequential filters that remove large particles first, followed by increasingly smaller ones.

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10:30 Saturday 6th July 2013

A1.36
Brownian motion shapes hydrodynamic hair cell mechanoreceptors

Mees Muller (Experimental Zoology, Netherlands), Coen Elemans (Sound Communication Group, Denmark) and Kier Heeck (Condensed Matter Physics, Netherlands)

In vertebrates, hair cell mechanoreceptors can be divided into statocyst receptors in which the hair bundles are deflected through the movement of connected particles, and hydrodynamic receptors in which fluid flow deflects the hair bundles. We studied the movements of hair bundles of the hydrodynamic group which are caused by thermal agitation i.e. Brownian Motion (BM). These movements may mask the adequate sensory movements of these receptors.
Hydrodynamic receptors also fall within two groups: high frequency sensors (HF, f>30 Hz) and low frequency ones (LF, f<30 Hz). HF-sensors are found in the lateral line and the cochlea, LF-sensors in the ampullae of the semicircular ducts. The HF-sensors are about 100–1,000 times more sensitive than the LF-sensors.
To explain this, we derived the equation of motion of hair bundles which is composed of thermal agitation-, hair bundle stiffness- and friction-terms. The calculated BM-movements were compared with measurements. It was revealed that the mechanics of a hair bundle form a low-pass filter with a corner frequency of 10–30 Hz for the white noise caused by BM. Therefore, HF-sensors are not masked or overloaded by BM in the concerning HF-bandwidth, whereas in the LF-bandwidth, HF-cells would be seriously overloaded. This explains the relative insensitivity of LF-sensors in the LF-bandwidth which is reflected in their stiffness and morphology. Another consequence is that the adequate stimuli have to be enlarged by guiding structures, i.e. the semicircular ducts. So the mechanics and morphology of these sensors are, at several size-levels, explained by consequences of thermal molecular fluid movements.

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10:45 Saturday 6th July 2013

A1.37
Chemical components of toe pad secretions of tree frogs and their role in wet adhesion

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Since tree frogs adhere by wet adhesion, the fluid secreted by their toe pads is critical to their function. It has not previously been analysed. The secretions are produced by specialized mucous glands located in the dermis yet they are epidermal in origin. The main force operating to achieve effective adhesion is capillarity. This force requires a thin layer of fluid with a low contact angle between fluid and surface (i.e. the fluid needs to wet the surface). However, the natural environment of many tree frogs contains a high proportion of hydrophobic plant leaves, which theoretically should reduce the capillarity by increasing the contact angle of the fluid, yet the frogs climb well on these surfaces.
This finding led us to think about the chemical components of these toe pad secretions. Using analytical techniques such as MALDI (matrix-assisted laser desorption/ionization) and GC-MS (gas chromatography–mass spectrometry), we found that the toe pad secretions of the tree frogs *Osteopilus septentrionalis* and *Litoria caerulea* include small proteins and lipids such as carboxylic acids and steroids. Carboxylic acids can act as surfactants, and thus might be responsible for the reduction of the contact angle on hydrophobic surfaces. This remains to be tested.
Using interference reflection microscope studies of toe pad secretions we showed that they wet both hydrophilic (glass) and hydrophobic (octadecyltrichlorosilane) (OTS) surfaces. It is thus apparent that surfactants within the secretions play a critical role in tree frog adhesion, by enabling high capillarity forces on hydrophobic plant surfaces.

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11:00 Saturday 6th July 2013

A1.38
Dominant role for capillarity in tree frog adhesion

W Jon P Barnes (University of Glasgow, United Kingdom), Walter Federle (University of Cambridge, United Kingdom) and Thomas Endlein (University of Glasgow, United Kingdom)

Tree frogs are thought to adhere by wet adhesion, using forces generated by the mucus that forms a fluid joint between pad and substrate. Using a two-dimensional force plate, we have recorded normal and shear forces from single toe pads of White's tree frogs (*Litoria caerulea*). In particular, we have investigated the extent to which adhesive forces depend upon capillarity.
In all trials, adhesive forces fell to low levels (<0.5 mN) when the toe pad was surrounded by a water drop, which was observed to abolish the meniscus that surrounds the toe pad and generates the capillarity forces (n=14). Without a water drop, adhesive forces could exceed 5 mN (~1 mN mm^{-2}). Is this reduction in adhesive force due to the absence of the meniscus or does it result from an increase in pad/substrate distance?
Although we were not able to estimate pad/substrate distance during force measurements, we repeated the procedure and observed the effects using interference reflection microscopy, which measures the thickness of a fluid layer. We showed that addition of a water drop resulted in a permanent increase in pad/substrate distance in 25% of cases, a temporary increase in 45% and no change in 30% (n=40). There was a substantial proportion of cases where the sharp fall in adhesive force could not be explained by an increase in pad/substrate distance.
We conclude that the loss of the meniscus is the critical feature, demonstrating the dominant role of capillarity in tree frog adhesion.

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11:15 Saturday 6th July 2013

A1.39
Sticking under wet conditions: The remarkable attachment abilities of torrent frogs (*Staurois guttatus*)

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Tree frogs climb smooth surfaces utilising capillary forces arising from an air–fluid interface around their toe pads, whereas torrent frogs climb in very wet environments near waterfalls. This study compares their adhesive capabilities, investigating possible adaptations for adhesion under wet conditions.
We challenged both frog species to cling to a platform which could be tilted from the horizontal to an upside-down orientation, testing the frogs on different levels of roughness and water flow. On dry, smooth surfaces, both frog species stayed attached to overhanging slopes equally well. In contrast, on wet and flooded surfaces, torrent frogs performed significantly better, even adhering under conditions where their toe pads were submerged in water, abolishing the meniscus that underlies capillarity. Both frog species not only used the contact area of their pads to adhere, but also large parts of their belly and thigh skin. In tree frogs, the belly and thighs often detached on steeper slopes, whereas torrent frogs increased the use of these areas as the slope angle increased.
Force per area measurements on the different skin parts revealed that forces from all these declined significantly in wet conditions, with only minor differences between the frog species. The superior abilities of torrent frogs were thus due to the large contact area used by torrent frogs on steep, overhanging surfaces. SEM images revealed slightly elongated cells in the periphery of the toe pads in torrent frogs, the channels between them facilitating drainage of excess fluid.

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11:30 Saturday 6th July 2013

A1.40

Locomotion dynamics in differently adapted fast-running arthropods

Tom Weihmann (Jena Institute of Sport Science, Friedrich Schiller University, Germany) and Lars Reinhardt (Jena Institute of Sport Science, Friedrich Schiller University, Germany)

Arthropods populate a vast multitude of terrestrial habitats. Each provides specific constraints for the animal's locomotor systems, which is reflected in various morphological adaptations. In the last decades spring-mass models were successfully applied to investigate COM oscillations of fast running arthropods in the sagittal and in the horizontal plane. The general applicability for all kind of fast, legged, terrestrial locomotion is widely accepted. Although elastic storage and reuse of movement energy provide several advantages, our examinations of ant and spider locomotion let raise doubts about the supposed universal validity of this scheme. By analysing the COM trajectories of the wood ant *Formica polyctena* during contact and swing phases of their alternating tripods we were able to demonstrate that the COM of these animals reaches its highest point at midstance, even at near maximum running speed of about 20 body lengths per second. This finding stands in contrast to the predictions of a sagittal spring mass model and indicates rather inverted pendulum mechanics. Additionally, spring-mass models predict specific frequency relations of rhythmically oscillating parameters. Thus, the cycle frequency of the legs at a given running speed should be mirrored by the rotational and translational degrees of freedom of the body. Our experiments on *Cupiennius salei* show, that in these large spiders the expected relations could not be found in slow or fast locomotion. Consequently, ants and spiders seem not to exploit the advantages of a running style typical for many other fast moving animals.

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11:45 Saturday 6th July 2013

A1.41

Jumping without slipping: Leafhoppers (Hemiptera: Cicadellidae) possess special tarsal structures for jumping from smooth surfaces

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Many Hemiptera are able to perform explosive jumps. As most hemipterans live on plant surfaces that can be smooth, we studied whether and how these insects are capable of taking off from smooth substrates. We compared jumps of froghoppers (*Philaenus spumarius*, Cercopidae) and leafhoppers (*Aphrodes bicinctus*, Cicadellidae) on smooth glass and rough sandpaper (30 µm asperity size). While *Philaenus* froghoppers on glass were unable to perform controlled jumps because their hind legs slipped extensively, *Aphrodes* leafhoppers took off normally. Froghoppers on glass jumped mainly upward and almost four times more slowly than on the rough substrate, whereas leafhoppers jumped with similar take-off angles and velocities on both substrates. High-speed microscopy of hind tarsi of *Aphrodes* jumping from glass revealed that 3–7 soft spurs per leg ('platellae', present in leafhoppers but not froghoppers) contacted the surface extremely briefly (~1 ms) during take-off. To test whether these structures can produce sufficient traction for forward jumps, we measured friction forces of individual tarsi on glass. When sliding slowly, shear forces were similar for pushing and pulling, and insufficient to explain the recorded low take-off angles. Only when the tarsi were pushed with velocities >1 mm/s did the contact areas increase strongly, and friction coefficients were high enough to explain the observed jumps.
Our findings demonstrate insects have evolved special tarsal structures for jumping from smooth surfaces, which achieve extraordinarily rapid control of attachment and detachment by combining directional adhesion with velocity-dependence.

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12:00 Saturday 6th July 2013

A1.42

Challenges of confined space locomotion – a case study using the American cockroach

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A composite exoskeletal system with an integrated array of sensors and muscles enables arthropods to locomote through the most restrictive environments. We found the tough yet compressible exoskeleton of the cockroach, *Periplaneta americana*, enabled the animal to run through confined spaces less than a third of its standing height (12–15 mm). We ran animals through a variable ceiling height rectangular tunnel at 4, 6, 9 and 12 mm heights. Surprisingly, animals ran within the vertically-restricted space with equal ease at high speeds (52.15±2.68 cm/s), only showing a decrease at the lowest height of 4 mm (12.56–2.45 cm/s, $p<0.01$). Animals maintained a tripod gait at all heights except 4 mm when feet often slipped on the surface (medium-grit sandpaper) and stereotyped leg trajectories were altered. Kinematic analysis revealed no significant change of leg cycling frequency (16.12±1.24 Hz, $p>0.05$) across the ceiling heights. However, cockroaches used significantly ($p<0.01$) shorter stride lengths at 4 mm. At the smallest ceiling height, animals chose a more serpentine path of travel and lost foothold traction in 40.2–3.49% ($p<0.01$) of the strides leading to significantly less effective propulsion. Although navigating confined spaces likely increases the normal load, remarkably animals showed limited adjustments of the tarsal (hind leg) extreme positions relative to the body centre-line, contrary to expectations. Insights obtained into strategies of high-speed, confined space navigation not only increases our understanding of the mechanical design principles of these organisms, but is also inspiring the development of novel robots.

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12:15 Saturday 6th July 2013

A1.43

Force distribution between multiple legs on the ground: Predictions from theory exemplified in cockroaches

Michael Günther (Universität Stuttgart, Germany) and Tom Weihmann (Friedrich-Schiller-Universität, Germany)

In many species and various gaits, there is often more than one leg on the ground during terrestrial locomotion, including climbing on arbitrary substrates. So from a mechanical point of view, a manifold of solutions for distributing forces among the legs is possible, that provide the same centre of mass movement in space. Here, we suggest a model approach, as reduced as possible, by which unique solutions can be predicted for an arbitrary number of legs. In that, the legs are assumed to be massless struts representing their axes from hip to ground. We demonstrate the applicability of our approach in a two-dimensional example: cockroaches climbing any slope in the sagittal plane between ±180°. We analyse the instant of midstance in which three legs are attached to the substrate. As part of the approach, a weighted sum of squared force components and joint torques is minimized. By varying the model parameters in our theoretical approach, we can tackle the question: What particular mechanical properties (e.g., internal loads, attachment parameters, etc) may have been crucial for the evolutionary design of the locomotion apparatus? Moreover, we give an example of how such a refined body design may enable then a powerful use of adjustable parameters for movement control. In cockroaches, it seems that reducing body height above ground is a control parameter suitable for reducing the danger of loosing grip when climbing steep slopes.

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13:15 Saturday 6th July 2013

A1.44

Comparison of GRF patterns between fins and limbs during terrestrial locomotion: Functional consequences for the evolutionary invasion of land

Sandy M Kawano (Clemson University, United States) and Richard W Blob (Clemson University, United States)

Paleontological examinations of the invasion of land by vertebrates suggest that limb-like appendages likely originated in aquatic environments, but direct comparisons of the functional consequences of using early limbs with digits, rather than fins, for terrestrial locomotion have not been performed. In addition, the hind limb played an important locomotor role in early limbed tetrapods, yet how the forelimb might have contributed towards hind limb-driven locomotion is unclear. To evaluate how fins and limbs facilitated the initial capacities for terrestrial locomotion in early stem tetrapods, we compared three-dimensional ground reaction forces (GRFs) produced by isolated pectoral fins of mudskipper fishes (*Periophthalmus barbarus*) during terrestrial crutching, and isolated footfalls by the forelimbs and hind limbs of walking tiger salamanders (*Ambystoma tigrinum*). These extant taxa exhibit numerous similarities to early tetrapods that make them appropriate functional models. Our results show that salamanders' forelimbs and mudskippers' pectoral fins exhibit similar magnitudes and timings of the peak net GRF; however, compared to salamanders' forelimbs, mudskippers' pectoral fins had a lower vertical component and more medial inclination of the GRF. Forelimbs and hind limbs of salamanders demonstrated numerous similarities at the peak net GRF (e.g., vertical and medial components), suggesting comparable capabilities for body support and a potentially important locomotor role of the forelimb during hind limb-driven locomotion in basal tetrapods. These data establish a foundation to compare the functional properties of fins and limbs for terrestrial locomotion, and help provide insight into the biomechanics that could have influenced the water-to-land transition in tetrapod evolution.

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13:30 Saturday 6th July 2013

A1.45

Combining fossil footprints, computer simulation, and experimental data to understand dinosaur locomotion

Peter L Falkingham (Royal Veterinary College, United Kingdom) and Stephen M Gatesy (Brown University, United States)

Dinosaur tracks offer a primary source of evidence for understanding not only the behaviours of individual taxa, but also the broader view of locomotor evolution through time. Track morphology emerges from the dynamic, coupled interaction between moving feet and substrate. Deep tracks, in which the foot has penetrated far into the sediment, record the most kinematic data. However, traditional methods of analysis (2D surface outlines) fail to capture the fundamentally volumetric nature of deep track morphology and formation essential to their interpretation.

In order to fully extract these data, we must visualize sub-surface foot movements and sediment responses within opaque substrates. To this end, we analysed guineafowl traversing a bed of poppy seeds, using X-ray reconstruction of moving morphology (XROMM) to reconstruct the 3D kinematics of the distal limb both above and below the surface for the first time. Guineafowl limb morphology and motion were incorporated into a discrete element simulation to produce virtual tracks in which the motion of individual particles could be dynamically observed. By combining experimental data with simulations, we were able to reconstruct foot motion paths and the reaction forces in the sediment of a 200 million year old fossil dinosaur track.

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13:45 Saturday 6th July 2013

A1.46

Lizards popping a wheelie: Bipedal running in Australian agamid lizards

Christofer J Clemente (University of Queensland, Australia)

Bipedal locomotion is widespread among various taxa. While the reason for bipedal locomotion in other taxa vary from energetic advantages to reassignment of the forearms to other uses, within lizards reasons for bipedal locomotion are still unclear. Recent modelling studies suggest bipedalism in lizards may be a consequence of a caudal shift in the body centre of mass, combined with quick busts of acceleration, together which causes a torque moment at the hip lifting up the front of the body. Lizards are essentially 'popping a wheelie'.

Some lizards appear to run bipedally sooner and for longer than might be expected from this simple model, and it has been suggested that these lizards have exploited the consequence of bipedal locomotion. However, it is unclear how common this exploitation is among lizards. Do some lizards try to run bipedally or do most simply run bipedally by accident?

We examined strides from 10 species of Australian agamid lizards. Five of the 10 species were capable of steady state bipedal locomotion. We estimated the empirical acceleration threshold for these species between quadrupedal and bipedal locomotion using logistic regression. Next we recorded the kinematics of the lizards stride, plus morphological positions of the body centre of mass, and input these into the model. Eight of the 10 species we able to beat the model, running bipedally at lower accelerations than predicted, suggesting that bipedalism in this group may convey some advantage, though what this advantage is remains the subject for future investigations.

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14:00 Saturday 6th July 2013

A1.47

Gradient limits and safety factor of Alpine ibex locomotion (on dam walls)

Carlo M Biancardi (University of Milan, Italy) and Alberto E Minetti (University of Milan, Italy)

Dam walls are like open laboratories useful to study the gradient limits of locomotion. A 64-m-high dam wall, where Alpine ibex use to climb searching for the salty exuded, was filmed at 0.2 fps. The straight slope of the wall was 123% from ground to 31 m, then 157% for 22 m, and then almost vertical.

In total 33 animals were filmed and their body mass estimated: large females (range 25–35 kg), medium females (15–25 kg) and kids (<15 kg). No large males were observed. The overall weighted average incline of their paths was 37% uphill and 46% downhill. They used to climb on zigzag routes and run down on more linear tracks. The gaits employed by the animals were walk and gallop. The steepest paths travelled by kids were 155% up and 157% down, the maximum height was 49 m, while their maximum estimated speeds were 2.6 ms^{-1} up and -4.2 ms^{-1} down. Medium: +143% and -157%; 49 m; +1.1 and -4.1 ms^{-1}. Large: +102% and -123%; 32 m; +0.7 and –1.0 ms^{-1}.

The climbing performance of Alpine ibex, in terms of speed and inclination, appeared to be negatively influenced by body mass, while the friction coefficient between their hooves and concrete was high, like rubber on solid surfaces. Protection against toppling depends on the slope and the ratio between the basal width and bCOM height. Therefore animals with shorter legs and lower bCOM, like females and kids, can negotiate steeper paths with a higher safety factor.

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14:15 Saturday 6th July 2013

A1.48
Are cheetahs special?

Tatjana Y Hubel (Royal Veterinary College, United Kingdom) and Alan Wilson (Royal Veterinary College, United Kingdom)

Cheetahs and greyhounds are known for their ability to run at exceptional high speeds and manoeuvre for prey capture. But how exceptional is that performance and how does it compare to the prey species they hunt?
Here we focus on the acceleration performance and associated muscle power output of two predator species cheetah and greyhound and two prey species, rabbit and hare. We measure the acceleration performance of predators using a GPS-IMU wildlife collar and calculate stride and stance average muscle power from velocity and acceleration data and stance times. Equivalent data for prey are derived from video and force platform measurements. These are compared with measures of muscle power made *in vitro* in our laboratory.
Pilot data for predator and prey acceleration performance and power were similar suggesting that the 'performance arms race' is constrained by other physiological factors and that factors such as detection and manoeuvring may be critical in determining hunt outcome.

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14:30 Saturday 6th July 2013

A1.49
Locomotor adaptations of Old World desert rodents

Talia Y Moore (Harvard University, United States), Alberto Rivera (Harvard University, United States) and Andrew A Biewener (Harvard University, United States)

Both bipedal and quadrupedal rodents are native to Old World deserts. Despite radically divergent locomotion, they share food source, predators, and activity period. While the quadrupedal rodents can be easily compared to the locomotion of other previously characterized quadrupeds, the bipedal rodents (*Jerboas* of the family Dipodidae) use a diverse set of jumps, hops, and skips that are difficult to compare to other bipeds. The trajectory of jerboas is also significantly more erratic than the sympatric quadrupeds.
In the lab, we used inverse dynamics to characterize the forces exerted by bipedal jerboas (*J. jaculus*) when jumping vertically, as well as the relative contributions of individual muscles and tendons to the jump. We collected trajectories of bipedal jerboas (*A. elater*) and sympatric quadrupedal jirds (*M. meridianus*) in the field to quantify the maximum performance and predictability of the escape behaviour of these species in natural conditions.
Understanding both how the behaviour is produced and exhibited in nature can reveal locomotor adaptation to predatory selective pressure and how these particular bipedal and quadrupedal desert rodents continue to live in sympatric equilibrium.

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14:45 Saturday 6th July 2013

A1.50
Ground reaction forces in racehorses

Zoe T Self (Middlesex University, United Kingdom), Andrew J Spence (Royal Veterinary College, United Kingdom) and Alan M Wilson (Royal Veterinary College, United Kingdom)

Ground reaction forces (GRF) are considered to be one of the limiting factors to maximum running speed, though have not been directly measured during high-speed, over-ground galloping in the horse. GRFs have been shown to be accurately estimated from duty factor at lower gaits but estimates have yet to be validated for high-speed galloping using force plate measurements. This study reports GRFs for multiple limbs, measured using force plates, across seven thoroughbreds during ridden galloping trials.
Results show peak vertical force values of similar magnitude to predicted v alues, though the distribution of bodyweight between the forelimbs and himdlimbs is 50%:50%, unlike the 60%:40% commonly stated for cursorial quadrupeds in the literature. Peak vertical forces ranged between 13.1–20.4 Nkg^{-1}, 12.4–17.2 Nkg^{-1}, 12.7–20.2Nkg^{-1} and 13.5–20.2 Nkg^{-1} for the non-lead hind, lead hind, non-lead fore and lead fore, respectively. The craniocaudal forces were low in magnitude and highly variable in comparison to the vertical forces, though appeared to concur with the observation that horses accelerate with the hindlimbs and brake with the forelimbs.
Understanding of GRFs enhances our knowledge on the biomechanics of athletic performance and can be utilized in the prevention and rehabilitation of racing injuries which may result from the high forces experienced by the musculoskeletal tissues of the limbs.

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15:00 Saturday 6th July 2013

A1.51
Be careful where you tread: Foot placement of horses during locomotion in a circle

Sandra D Starke (Royal Veterinary College, United Kingdom), LeeAnn J Kaiser (Michigan State University, United States), Narelle C Stubbs (Michigan State University, United States), Thilo Pfau (Royal Veterinary College, United Kingdom) and Hilary M Clayton (Michigan State University, United States)

The mechanics of navigating a circle or turn has long been a fascinating research question. Previous work on locomotion around bends has largely focussed on limits to performance in bipeds and quadrupeds. In horses lunged on circles, changes in limb angles and ground reaction forces at various gaits as well as vertical displacement asymmetry at trot have been documented. These results confirm that, as one would expect, animals have to change the way they move to get around bends.
However, to date a simple question remains: how does a quadruped actually place its feet when moving on a circle? This topic is especially of interest for biologically-inspired robotics. As part of our collaborative project into the mechanics of circular locomotion, we used a 10-camera optical motion capture system (Motion Analysis Corp) to document foot position of horses in 3D space at different gaits. Horses were lunged on a 3 m radius circle both at walk and trot. For comparative purposes, horses were also led along a straight line at the same two gaits. We investigated location and timings of foot placements in the horizontal plane. These data allow us an insight into the principles of turn navigation and the individual strategies displayed by different subjects.
Further, we examine the effect of turn direction (left rein *versus* right rein) on foot placement in the framework of laterality.

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15:40 Saturday 6th July 2013

A1.52
The basic mechanics of quadrupedal gaits

James R Usherwood (Royal Veterinary College, United Kingdom)

Quadrupeds show a fascinating range of gaits, both between species and across speeds. Accounting for the selection of these gaits, and understanding them within the context of mechanics, body form and locomotory requirements remain challenging. Current extreme reductionist models provide a range of insights, but fail to account for many aspects of gait selection.
Here, I extend point mass models (which predict exactly evenly spaced footfalls, and cannot account for trotting) to include two additional levels of complexity: points of force application on the ground being distributed (because of a finite back length); and the forces are allowed to apply torques about the centre of mass (because of a finite pitch moment of inertia). In effect, the analyses and models treat a quadruped as a stiff table.
This approach successfully accounts for why horses gallop with only a gathered aerial phase (and frogs extended). However, if the body geometry does not vary with speed, no account is made for a transition from pronking to pitching gaits (or trotting to galloping) with increasing speed. Indeed, the energetic costs of non-pitching gaits (pronking, trotting and pacing) are predicted to be independent of speed, while pitching gaits (bounding, frog-hopping, galloping, etc) are predicted to increase with speed. So, while the model provides novel and, in retrospect, intuitive insight into the footfall timing and direction of forces during pitching gaits, it also predicts a gallop to trot transition with increasing speed. Likely limitations of the model assumptions will be considered.

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15:55 Saturday 6th July 2013

A1.53
Perturbed bipedal running: Speeding-up or bouncing high?

Yvonne Blum (Royal Veterinary College, United Kingdom) and Monica A Daley (Royal Veterinary College, United Kingdom)

Our goal is to identify control strategies used by birds to achieve stable and robust locomotion in uneven terrain. We want to investigate how touch-down conditions, which are determined by swing leg control strategies, influence stance dynamics during steady state running and in the presence of a permanent drop and a pothole. We hypothesize that, depending on the encountered ground height disturbance (drop *versus* pothole), birds use different swing leg strategies in the first step of the drop/pothole.
In a previous study we have shown that guinea fowl, when running a step down, transfer the additional potential energy into kinetic energy by speeding up during the drop step, and then actively remove the added energy by slowing down in the subsequent steps. When encountering a pothole, however, different strategies are possible: They could: i) speed up and then launch themselves back onto the original ground height; ii) run with a more extended leg configuration; or iii) maintain a higher bouncing gait throughout the pothole.
Avian running trials were conducted on a runway, and dynamics and kinematics of eight birds (guinea fowl, *Numida meleagris*) were recorded. We had three experimental setups, consisting of a flat runway, a ramp with a 6 cm drop and a 6 cm pothole.
To underpin our hypothesis, we test different swing leg control strategies based on a simple, planar spring mass model with a leg actuator and compare the simulation results with the observed experimental data.

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16:10 Saturday 6th July 2013

A1.54
Limb mechanics during steep downhill locomotion in humans

Peter Aerts (University of Antwerp, Belgium), Pieter Fiers (University of Antwerp, Belgium), Ine Van Caekenberghe (University of Antwerp, Belgium) and Dirk De Clercq (University of Ghent, Belgium)

Running down steep slopes is a demanding task. Gravity constantly tends to accelerate the body in the direction of motion and running at a constant pace requires leg muscles to do negative (eccentric) work equivalent to the input from gravity (with complaints about soreness in the limb extensors as a result). This study focuses on whole-body and lower limb mechanics (kinematics and kinetics) during steep downhill running in order to reveal how the human musculoskeletal system copes with this demanding task.
Subjects ran down a 14.3° inclined instrumented runway (16.5 m x 2.5 m) at preferred and selected speeds. Analysis showed differences in spatiotemporal parameters (minimizing floating phases, hence the unrestrained energy gain, of the running strides) and in lower limb mechanics between level and downhill running.
The main results indicate that maximal knee extensor moments are comparable to these observed in level running but that maximal negative work and negative power at the knees (contrary to findings for peak power on weaker slopes[1]) are larger during downhill running. Moreover, hip muscles must work harder during downhill running, likely to stabilize the upper body.
It is very often observed that humans spontaneously adopt a bipedal galloping gait when moving fast downhill. According preliminary results, this gait change may be an alternative for minimizing floating phases during running, to reduce the loading at the level of the knees.
1. Buczek, F. & P. Cavanagh (1990). *Medicine and Science in Sports and Exercise* 22, 669–677.

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16:25 Saturday 6th July 2013

A1.55
Impulsive ankle push-off powers leg swing in human walking

Daniel Renjewski (Oregon State University, United States), Susanne Lipfert (Human Motion Engineering, United States), Michael Günther (Universität Stuttgart, Germany) and André Seyfarth (TU Darmstadt, Germany)

Rapid unloading and a peak in power output of the ankle joint have been observed during push-off in human walking. Model based studies have hypothesized this push-off to cause redirection of the body's centre of mass just before touch-down of the contra-lateral leg. Muscle work has been discussed to power this catapult-like mechanism. However, findings of isometric muscle operation of the ankle extensors during stance phase contradict this assumption.
We used kinematic and dynamic data of human walking collected at speeds between 0.5 and 2.5 m/s for a comprehensive analysis of push-off mechanics. Our results show a larger impulse for the trailing leg than for the remaining body during push-off. The buckling knee joint inhibits transfer of power from the ankle to the remaining body and at the same time enables rapid propulsion of the trailing leg.
In our study we identified the mechanism and functional context of the ankle push-off, namely a catapult accelerating the trailing leg into swing. With the ankle extensors showing passive elastic behaviour, our findings indicate power amplification by accelerating a small mass without adding muscle work. Therefore, it appears that swing initiation profits from an impulsive ankle push-off. This characteristic behaviour in human walking expands our understanding of the fundamental mechanics.

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16:40 Saturday 6th July 2013

A1.56
An energetic basis for obstacle traversal behaviour in humans presented with a single hole of variable length and depth

Katherine Daniels (University of Bristol, United Kingdom) and JF Burn (University of Bristol, United Kingdom)

Both natural and man-made terrestrial environments commonly exhibit spatially localized reductions in ground height (holes). If a hole is to be traversed without re-routing, two options (termed traversal strategies) are available: to step down onto the base of the hole and then up out of it or to step over the hole. We proposed that a systematic choice between traversal strategies would be made on the basis of energetic cost. Human subjects walked along a straight 13 m track into which was set a hole that could be varied in depth (10–50% leg length) and length (30–70% leg length). Kinematic data were recorded using optical motion capture and combined with published anthropometric data to calculate mechanical energy of obstacle traversal. All subjects were able to use both traversal strategies over the range of hole dimensions used for the experiment.
We showed that a traversal strategy was systematically selected based on both the length and the depth of the hole. The strategy selected was found generally to be that which minimized the mechanical energy cost of transport for the traversal. A divergence in kinematics dependent on the strategy to be implemented was observed towards the end of the ultimate step prior to reaching the hole. The study supported the hypothesis that the strategy selection for locomotion over holes in the ground is energetically optimized within the range of dimensions investigated.

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16:55 Saturday 6th July 2013

A1.57
Effects of body weight on foot grip force

Junichiro Yamauchi (Graduate School of Human Health Sciences, Tokyo Metropolitan University, Japan)

Because the foot bears body weight as it carry the body through daily activities, it plays an integrative role in controlling the posture and movements. There are numerous studies on muscle functions of lower limbs; however, only few studies have investigated how foot generates force during standing. Many physical activities are performed with a standing position, thus it is important to understand the foot function during standing position. We have shown that maximum isometric foot grip force was significantly higher during standing than sitting position. Therefore, the present study was to investigate how body weight affected to the maximum isometric foot grip force.
Twenty-two healthy young individuals were measured the maximum foot grip force on the dynamometer. For the measurement of maximum isometric foot grip force, subjects exerted force with maximum effort for ~3 seconds on a foot grip dynamometer in sitting, standing, standing with 50% of body weight (BW) and standing with 10 kg of BW positions. Measurements were repeated three times with at least one-minute rest period between bouts, and the averaged value among the measurements was used.
Foot grip forces in the standing position was significantly higher than in the sitting position; however, after body weight was reduced by unweight system, foot grip force in standing position was decreased and not much different from the sitting position. The present results indicate that the ability to generate foot grip force in standing position was somehow regulated by own body weight.

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17:10 Saturday 6th July 2013

A1.58
Migrating ibises benefit from wingbeat tuning in formation flight

Steven J Portugal (Structure Motion Lab, Royal Veterinary College, United Kingdom), Tatjana Y Hubel (Structure Motion Lab, Royal Veterinary College, United Kingdom), Johannes Fritz (Waldrappteam, Austria), Alan M Wilson (Structure Motion Lab, Royal Veterinary College, United Kingdom) and James R Usherwood (Structure Motion Lab, Royal Veterinary College, United Kingdom)

The distinctive V formation of bird flocks has long intrigued researchers and continues to attract both scientific and popular attention. The well held belief is that such aggregations give an energetic benefit for those birds which are flying behind another bird, through using the regions of upwash generated by the wings of the preceding bird. Though much theory has been applied to describe the precise positioning that birds should adopt within a V to maximize such energy savings, a definitive answer as to the aerodynamic implications of these formations has proved elusive. This has been, in part, due to the lack of suitable technology limiting the study of such behaviour in free-flying birds, and the shortcomings of applying fixed-wing theories to flapping flight. We show that individuals flying within a V flock position themselves in aerodynamically optimum positions, predicted by fixed-wing aerodynamic theory.
We demonstrate that when in V positions, birds exhibit wingtip-path coherence, flapping spatially in phase, which enables upwash capture to be maximized throughout a full flap cycle. In contrast, when birds fly immediately behind another bird, wingtip-paths are in spatial antiphase. This reduces the adverse effects of downwash for the following bird. This accomplishment was previously not thought possible because of the intricate flight dynamics and sensory feedback that would be required to perform such a feat.
We conclude that the intricate mechanisms involved in V formation flight indicate remarkable awareness of, and ability to, either sense or predict the spatial wake structures of nearby flock-mates.

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Poster session – Thursday 4th July 2013

A1.59
The adhering abilities of tree frogs on rough surfaces

Niall Crawford (University of Glasgow, United Kingdom), Thomas Endlein (University of Glasgow, United Kingdom), Mathis Riehle (University of Glasgow, United Kingdom) and W Jon P Barnes (University of Glasgow, United Kingdom)

Tree frogs possess highly adapted toe pads, which secrete a mucus that allows them to adhere through a combination of capillary and viscous forces. This, combined with specialized cell morphology, culminates in the toe pads producing impressive adhesive and frictional forces. However, most experiments testing the pads have thus far have been conducted on smooth surfaces; although these highlight the animal's capabilities, tree frogs are more likely to encounter rough surfaces in nature, such as a branch or a leaf. Here, the abilities of the toe pads of White's tree frogs (*Litoria caerulea*) are tested on rough surfaces for the first time. Adhesive and friction forces were recorded from individual toe pads using a 3D force transducer, with a motorised stage mimicking steps taken by the pad. Multiple surfaces were created from embedding resin and PDMS, with surfaces of varying roughness. The performance of the frogs' toe pads varied for each surface, with adhesion being reduced by the larger scale roughness, and friction forces increasing on all rough surfaces. Performance on smaller scale roughness doesn't vary from that on a smooth surface. These results indicate that there is a roughness scale where tree frog adhesion performs least effectively, while smaller levels of roughness do not detrimentally affect their pads.

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Poster session – Thursday 4th July 2013

A1.60

The functional morphology of the Northern clingfish feeding on limpets

Krijn B Michel (University of Antwerp, Belgium), Dylan K Wainwright (University of Washington, United States) and Adam Summers (University of Washington, United States)

The Northern clingfish (*Gobiesox maeandricus*) readily feeds on different species of limpet. Limpets are difficult prey, with tough calcium carbonate shells adhering closely to the substrate, few predators feed on limpets.
G. maeandricus manage to overcome the challenges of feeding on limpets by using a unique feeding technique capable of removing limpets from the substrate. Through the use of high speed recording and micro CT-scan we answered the following questions:
(1) What are the primary structures involved in this unique prey capture process?
(2) How is the clingfish morphology adapted to perform these movements?
(3) How would the force needed to dislodge limpets be generated?
The feeding apparatus of *G. maeandricus* is modified to be able to force the teeth of the lower jaw as a wedge between the limpet shell and the substrate. The suspensorium, neurocranium and vertebral column are all well ossified with broad interconnections oriented to support anterior movement of the lower jaw. By using the pectoral suctorial organ as a fulcrum, the epaxial musculature can be used to force the cranium forward through the vertebral column. The hypaxial muscles may be used to elevate the entire skull, including lower jaw. We hypothesize that the pectoral suctorial organ is of crucial importance for clingfish to be able to generate enough force to feed on limpets.

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Poster session – Thursday 4th July 2013

A1.61

Hand postures during vertical clinging and grasping: Implications for digit length in primates

Laura E Johnson (Ross University School of Medicine, Dominica) and Daniel Schmitt (Duke University, United States)

The presence of a grasping clawless hand presents biomechanical challenges when clinging to a vertical substrate and raises questions about the loss of claws in the primate lineage. Few experimental data have been collected to assess how extant primates solve the problem of clinging to vertical supports without claws.
Here we begin to address the question by hypothesizing that hand postures are influenced and constrained by substrate size. Individuals of eight primate species from the Duke Lemur Center, ranging in size from 150–4,000 g with differing locomotor modes, were prompted to vertically cling and grasp on clear PVC pipes of three sizes and their postures were recorded with two cameras.
The angle of the hand, defined between the tip of digit two, the wrist, and the tip of the pollex were quantified. Additionally, the position of the pollex relative to the substrate and other digits was assessed.
We found that as substrate size increased, individuals would move the pollex close to the other digits, removing the hand from a grasping posture where the pollex and digit two are opposed.
When analysed in the context of theoretical models of vertical clinging, these data can explain why many arboreal primates have reduced or absent pollices.

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Poster session – Thursday 4th July 2013

A1.62

Sexual dimorphism of the head and its effect on swimming performance in the maternally mouthbrooding cichlid *Oreochromis niloticus*

Sam Van Wassenbergh (Ghent University, Belgium), Nuno Zavattieri Potes (Ghent University, Belgium) and Dominique Adriaens (Ghent University, Belgium)

Females of the Nile tilapia (*Oreochromis niloticus*) can take an impressive amount of fertilized eggs into their mouth during brooding. Presumably as an adaptation for mouthbrooding, adult cichlids evolved a sexual dimorphism of the head. Here, we quantified the three-dimensional shape and size differences between the external head surfaces of male and female Nile tilapias, and tested whether these differences have an effect on the hydrodynamic performance of the head serving as a bow during swimming.
The external surfaces of six specimens of each gender were captured by 3D laser scanning, and hydrodynamics were simulated using computational fluid dynamics. After scaling all scans to a given volume of the body (excluding the head), the external volume of the head was 14% higher in females compared to males due to an increase in length and width of 7% and 4%, respectively. The height of the head did not differ significantly between the sexes. The calculated drag force increased by about 5% for a given body volume when swimming steadily at 0.5 meters per second.
This analysis suggests that, by predominantly increasing the size of the head along the direction of the water flow (i.e. head length), the hydrodynamic cost of the increase in head volume remains limited.

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Poster session – Thursday 4th July 2013

A1.63

Attachment device of male diving beetles: Functional morphology and dynamics

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Male diving beetles have specialized palettes to adhere onto female's elytra for mating underwater. Two types of adhesive setae are found on the palette: sucker-like circular setae, and spatulate setae with proximal sucker and parallel channels extended distally. Measurement of museum specimen showed that palette size of either type increased with body size, but those with spatulate setae tended to be smaller. To examine whether spatulate setae have better adhesive performance to compensate for smaller total palette area, we compared attachment ability and functioning mechanisms of circular setae from *Hydaticus pacificus* and spatulate setae from *Cybister rugosus*.
Adhesive force generated per setal area was comparable between two types of setae. Total adhesive force estimated for one palette of *H. pacificus* was about 50 BW (body weight), greater than 15 BW for *C. rugosus*. In both types, adhesion and shear resistance increased with load, but adhesion of spatulate setae had more sensitive response. While comparing the effects of detaching velocity on adhesion, different trends appeared: the circular setae performed best at low velocity; in contrast, the spatulate setae generated greater adhesive force with increasing velocity. We used a pipe flow model to explain this velocity-dependent adhesion. In spatulate setae, the lateral resistance was greater than that measured along the channel directions, facilitating preferred setal alignment under load. Compared to circular setae, more commonly found in male diving beetles, the velocity-dependent adhesion and direction-dependent shear resistance of spatulate setae make them unusual adjustable attachment device.

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Poster session – Thursday 4th July 2013

A1.64

Measurement of ground reaction force of ant using MEMS force plates

Hidetoshi Takahashi (University of Tokyo, Japan), Kiyoshi Matsumoto (University of Tokyo, Japan) and Isao Shimoyama (University of Tokyo, Japan)

The biomechanics of insect running have been widely studied due to its unique characteristics of step pattern. To investigate the running step pattern, the analysis of the ground reaction forces of insect's legs is important. Conventional studies presented a triaxial force sensor, and the ground reaction force of ant's legs was measured. However, the presented sensor was too large to be arrayed and to measure a sequence of steps of multi-legs.

In this research, we propose micro electromechanical systems (MEMS) force plates to measure vertical and anterior directional forces generated on multi-legs of ants (*Formica japonica*) simultaneously. The body length and weight of the ant were 5 mm and 3 mg, respectively. The plates were designed to be 1 mm × 0.75 mm in size, and to be arrayed in 1.25 mm pitch. The fabricated plates were able to measure both directional forces ranging to 20 μN with resolution of 1 μN. We measured the forces of the multi-legs of one side simultaneously. The total grand reaction forces of six legs were calculated from the time-normalized data. The averages of the grand reaction forces were 29 μN in vertical direction and -0.7 μN in anterior direction, respectively, which were corresponding to the kinematic moment. The grand reaction forces remained within the average ±10% and ±7%, respectively, in one running cycle. The measured value was thought to be a characteristic force for a running ant.

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Poster session – Thursday 4th July 2013

A1.65

Bite matters: Sexual dimorphism in the mandibular apparatus of a caviomorph rodent (C*tenomys australis*)

Aldo I Vassallo (Universidad Nacional de Mar del Plata, Argentina), Federico Becerra (Universidad Nacional de Mar del Plata, Argentina), Matías Mora (Universidad Nacional de Mar del Plata, Argentina) and Adrià Casinos (Universitat de Barcelona, Spain)

The South American rodent *Ctenomys* uses both forelimbs and incisors for digging. Incisors are also used in aggressive encounters between males, having *Ctenomysa polygynous* mating system. The effectiveness of a bite, in inflicting physical injury, depends on the robustness of teeth and the force of jaw muscles. The sand dune tuco-tuco, *Ctenomys australis* (mean body mass 350 and 450 g, in females and males, respectively) digs complex burrows in coastal dunes of Buenos Aires province.

We analysed possible sexual dimorphism in the mandibular apparatus of that species assessing allometric trajectories of both sexes. Bite force (BF) at the incisors was measured *in vivo* using a force transducer, in field trapped individuals [mean BF in males (n=21) 69.5 N; mean BF in females (n=34) 53.5 N; ANOVA p = 0.001]. ANCOVA results (p = 0.39 and p <0.001, respectively) showed that differences were not related to sex but to body size. On specimens of both sexes cross-sectional area (CA), second moment of area (I) and polar moment (J) of the incisors were calculated to assess strength. Males showed significantly higher allometric coefficients for CA and I, and also for J (ANCOVA: p = 0.026, p = 0.04 and p = 0.065, respectively), although in this case without statistical differences. This fact suggests the existence of sexual dimorphism in incisor's traits beyond sex body size differences.

Dimorphism in the mandibular apparatus of *C. australis* could be the outcome of sexual selection associated to the polygynous mating system of the genus

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Poster session – Thursday 4th July 2013

A1.66

'Heel' pads of stick insects (*Carausius morosus*) are pressure-sensitive friction pads with little adhesion

David Labonte (University of Cambridge, United Kingdom) and Walter Federle (University of Cambridge, United Kingdom)

Many insects possess attachment pads on their feet that enable them to climb on various surfaces. Indian stick insects (*Carausius morosus*) have two distinct types of pads on each leg, tarsal 'heel' pads (euplantulae) and a pre-tarsal 'toe' pad (arolium). Here we show that euplantulae are specialized 'friction pads'.

Single-pad force measurements showed that the friction force of euplantulae increased with normal load, while adhesion remained small in relation to body weight. This load-dependence is based on micron-sized microtrichia covering the surface of the euplantulae. Reflected-light microscopy revealed that both higher normal load and higher shear forces increased the number of microtrichia in contact, and caused some individual microtrichia to change from tip to side contact. Both mechanisms are reversible, increasing the real contact area of the pad and thus its shear resistance under load, but causing only little adhesion, as the elastic energy stored in the deformed microtrichia helps to break contacts during detachment.

As a result, euplantulae showed high friction coefficients (>1), despite low macroscopic adhesion. Our results show that stick insect friction pads produce high traction when pressed against the substrate, but at the same time allow effortless detachment.

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Poster session – Thursday 4th July 2013

A1.67

The detachment mechanism and three-dimensional ground reaction forces of tree frog toe pads during walking and climbing

Diana Samuel (University of Glasgow, United Kingdom), Thomas Endlein (University of Glasgow, United Kingdom), Jon Barnes (University of Glasgow, United Kingdom) and Mathis Riehle (University of Glasgow, United Kingdom)

Tree frogs adhere to surfaces using a temporary adhesive mechanism known as 'wet adhesion'. In keeping with the principles dictating temporary adhesives, this should enable the frog to both 'stick' adequately and detach its adhesive toe pads effortlessly. To investigate this, we have developed a system that allows us to simultaneously observe toe pad contact area and acquire ground reaction forces, during walking and climbing locomotion.

A three-dimensional force plate is embedded in a 'runway' that can be oriented in either a horizontal or vertical manner. The plate consists of a small square of glass, illuminated by light-emitting diodes. Due to the principles of total internal reflection, the area of the toe pad becomes visible upon contact with the glass. Each experiment is concurrently recorded from ventral and dorsal perspectives using high-speed cameras, and these recordings are synchronised with force data acquisition. Therefore, this system enables us to correlate changes in pad area with force.

Preliminary data suggest that White's tree frogs (*Litoria caerulea*) do need to exert a relatively small amount of force to detach their toe pads, and this is often preceded by a forwards 'push' – potentially relieving the shear stress on the pad and initiating peeling. The results also highlight apparent differences in leg function between forelimbs and hindlimbs, particularly during climbing, such as the hindlimbs compensating for the pitching moment exerted on the forelimbs. This system thus affords us an opportunity to explore the dynamic aspects of the tree frog adhesive system in more detail.

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Poster session – Thursday 4th July 2013

A1.68
Constraints to muscle performance provide a novel account for the scaling of posture in terrestrial animals

James R Usherwood (Structure and Motion Lab, Royal Veterinary College, United Kingdom)

Larger terrestrial animals tend to support their weight with more upright limbs. This makes structural sense, reducing the loading on muscles and bones, which is disproportionately challenging in larger animals. However, it does not account for why smaller animals are more crouched; instead, they could enjoy relatively more slender supporting structures or higher safety factors.
Here, an alternative account for the scaling of posture is proposed, with close parallels to the scaling of jump performance. If the costs of locomotion are related to the volume of active muscle, and the active muscle volume required depends on both the work and the power demanded during the push-off phase each step (not just the net positive work), then the disproportional scaling of requirements for work and push-off power are revealing.
Larger animals require relatively greater active muscle volumes for dynamically similar gaits (e.g. top walking speed) – which may present an ultimate constraint to the size of running animals. Further, just as for jumping, animals with shorter legs and briefer push-off periods are challenged to provide the power (not the work) required for push-off. This can be ameliorated by having relatively long push-off periods, potentially accounting for the crouched stance of small animals.

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Poster session – Thursday 4th July 2013

A1.69
The physiological determinants of the scaling of muscle power output during escape swimming in scallops

Thomas R Neil (University of Leeds, United Kingdom), Marion Kauffmann (University of Leeds, United Kingdom) and Graham N Askew (University of Leeds, United Kingdom)

The scaling of muscle power output is crucial to understanding size-related differences in animal locomotion. It has been hypothesized that muscle mass-specific power output should scale negatively with body mass (M_b) according to either geometric or elastic similarity (as $M_b^{-0.33}$ or $M_b^{-0.125}$, respectively). To test these hypotheses we studied escape swimming in scallops, a system in which natural selection has likely operated on the structural design of the muscle in favour of a high power output. We determined muscle mass-specific power output in vivo during escape swimming in scallops that covered a range of body masses by measuring the pressure pulses and the change in volume (Power output = pressure ´ flow rate).
Muscle mass-specific power decreased with increasing body mass, scaling as $M_b^{-0.119}$. These results are consistent with scaling of muscle mass-specific power according to elastic similarity rather than geometric similarity. In order to gain insight into the physiological determinants of this scaling relationship we characterized the contractile properties of the adductor muscle in vitro. Isometric twitch characteristics and isotonic force–velocity relationships were determined for the muscles, providing information on:
(1) the scaling of the deactivation rate;
(2) the scaling of the curvature of the force-velocity relationship; and
(3) the scaling of V_{max}.
This integrative approach of *in vivo* and *in vitro* physiological measurements will add to our general understanding of how animals use muscle to power locomotion and the consequences of differences in animal size.

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Poster session – Thursday 4th July 2013

A1.70
Morphing wings of a hovering hummingbird

Hiroto Tanaka (Chiba University, Japan), Ikuo Kitamura (Chiba University, Japan), Masateru Maeda (Chiba University, Japan), Toshiyuki Nakata (University of Oxford, United Kingdom) and Hao Liu (Chiba University, Japan)

Hummingbird wings are composed of radially distributed multiple feathers, which can lead to passive wing deformation in flapping flight and hence some variation in wing area. While the effects of the flexible wing deformation on hovering aerodynamics of insects have been a hot topic recently, less attention has been paid to the time variation in the wing area of the feathered wing and its correlation with flapping-wing aerodynamics. In this study we present a study on quantifying such feathered wing deformations of a hovering hummingbird, *Amazilia amazilia*, with a specific focus on the variations in wing area.
Hovering flight of the hummingbird was filmed and recorded with four synchronized high-speed video cameras in a conservatory in Tama Zoological Park, Tokyo, Japan. The wing outline and feather shafts were first manually traced for each captured image and three-dimensionally reconstructed. Then a smooth curved surface was fit to the reconstructed points to create a 3D wing model.
Ours results showed that the wing surface area varied dynamically in flapping flight, achieving a maximum of 20% in a complete wing beat cycle, which was observed in the mid-down-stroke. By comparing the spreading angles of the feather shafts and the wing areas, we found a specific correlation between the time-varying wing area and the expansion of the feathers. Our results suggest that the dynamic wing deformation, in particular the variation in wing area, may be responsible for enhancing the aerodynamic performance in hummingbird hovering.

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Poster session – Thursday 4th July 2013

A1.71
The roll of abdominal motions in the free flight of a hawkmoth

Ryusuke Noda (Chiba University, Japan), Masateru Maeda (Chiba University, Japan) and Hao Liu (Chiba University, Japan)

It is known that most of the insects have flexible structures on their bodies, which deform during flapping flight. However, most of the previous computational studies of insect flights have treated the insect body as rigid and less attention has been paid to the body deformation. In this study, to evaluate how the body deformation of a hawkmoth (*Manduca sexta*) affects flight stability, we have developed a fluid-structure interaction (FSI) simulator by coupling a flexible body dynamics solver for three-dimensional flexible beams with an in-house Navier-Stokes solver. The joint flexibility effect was studied by changing the Young's modulus at the joints and our results indicated that the body deformation does extend influence on the variation of the body attitudes. By incorporating a simple PID-based flight control system into the FSI simulator, we observed that our model could achieve a stable hovering flight of the hawkmoth with both flexible and inflexible body models while the converged wing kinematics showed slight differences. Furthermore, we investigated the effects of active abdominal motions on the transition from hovering flight to forward flight, which also pointed to the importance of the body deformation.

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Poster session – Thursday 4th July 2013

A1.72
A miniaturized force plate system for use in rodent exercise wheels

Benjamin JH Smith (Royal Veterinary College, United Kingdom), Lottie Cullingford (Royal Veterinary College, United Kingdom) and James R Usherwood (Royal Veterinary College, United Kingdom)

Measuring the force applied to the ground by an animal's feet during locomotion is a common technique for analysing gait dynamics. However, most commercially available force plates are designed for humans or large animals, and are therefore not suitable for animals such as mice due to sensitivity limitations and the tendency of small animals to run in short bursts rather than at a steady speed.

We present a miniature force sensing platform for use in rodent exercise wheels and small treadmills, based on Hall sensor technology. The advantage of using Hall sensors over strain gauges or piezo sensors is that the electronic components can be mounted below the wheel or treadmill, eliminating the need for a slip ring to provide power to the sensor; the system is also much less sensitive to temperature variations or static interference. The result is a compact and robust system which can be easily mounted in exercise wheels of various designs and sizes. This system can also be used to measure the speed of the wheel, and hence determine the speed of the animal.

The sensor was tested in a range of commercially available rodent exercise wheels to determine the ground reaction forces during locomotion and the speed of locomotion of laboratory mice. This allowed measurement of these quantities during voluntary rather than forced running, and over a longer time period than can be observed on open ground.

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Poster session – Thursday 4th July 2013

A1.73
Climbing strategies in ants

Lars Reinhardt (Motion Science Institute of Sport Science, Friedrich Schiller University, Germany)

Climbing is an everyday challenge for insects because they always have to move in a highly structured environment. However, the strategies how these fast runners cope with slopes are largely unknown so far. In order to better understand these strategies we have studied continuous upward locomotion of the wood ant species *Formica polyctena* at substrate angles from 0° to 90°.

We measured the 3D kinematics of the main body segments and the legs, and, for the first time, the ground reaction forces of all legs in a self-developed test site with an integrated highly sensitive three-dimensional miniature force platform. Force measurements of comparable fast insects have only been published for much larger cockroaches at two substrate angles (0° and 90°). Wood ants do not make any slope dependent modifications in posture and stride pattern. Even at steep climbs movement kinematics cannot be distinguished from flat locomotion and still appears very dynamic.

As might be expected, there are major changes in the ground reaction forces. Thus, when climbing, the front legs generate high pulling forces directed towards the body in anterio–posterior and lateral direction. The significantly lower normal forces are also directed towards the body and have approximately the same amount as the opposite directed normal forces of the hind legs. At steep inclines, the propulsion provided by these legs is low compared to level locomotion.

Our results show that ants use a similar climbing strategy to cockroaches.

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Poster session – Thursday 4th July 2013

A2 – NEURO-ETHOLOGY AND BIOMECHANICS OF ACOUSTIC COMMUNICATION IN VERTEBRATES

Organized by: Coen PH Elemans (University of Southern Denmark) and John M Ratcliffe (University of Southern Denmark)

A2.1
Vertebrate innovations in the neural basis of vocal-acoustic mechanisms: Developmental origins and evolutionary patterns

Andrew H Bass (Cornell University, United States)

Acoustic signalling behaviours are widespread among the majority of living fishes and tetrapods. Here, I will discuss the developmental and evolutionary origins of the neural basis for novel innovations in vocal–acoustic mechanisms, integrating across behavioural, neurophysiological and morphological levels of analysis. The focus will be on two seemingly unrelated motor systems, vocal signalling and movement of pectoral appendages, that is, fins and forelimbs.
The available developmental evidence for fishes, amphibians, birds and mammals indicates that vocal behaviour and its central nervous system basis are ancestral vertebrate characters. Surprisingly, recent studies in fishes show that the premotor–motor circuitry for pectoral appendages that function in both locomotion and acoustic signalling in fishes and tetrapods, develops in the same neuroepithelial compartment as that for vocalization.
As will be discussed, these new findings imply that the neural basis for vocal and pectoral behavioural phenotypes, inclusive of roles in acoustic communication and forelimb gestures, share developmental origins that first appeared among fishes.

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11:05 Wednesday 3rd July 2013

A2.2
Evidence against power amplification in the mammalian inner ear

Marcel Van der Heijden (Erasmus MC, Netherlands) and Corstiaen PC Versteegh (Erasmus MC, Netherlands)

Sound evoked travelling waves in the cochlea are widely believed to be amplified by a process of cycle-by-cycle power injection by outer hair cells. We performed an analysis of recent cochlear-mechanical data, both from others and from our own lab. We found that the power of the wave does not grow, and never exceeds the acoustic power delivered by the stimulus.

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11:50 Wednesday 3rd July 2013

A2.3
Superfast and ultrasonic: Design constraints on echolocation in bats

John M Ratcliffe (University of Southern Denmark, Denmark)

Bat biosonar continues to provide insight into how bats and other animals build and update internal representations of the outside world. Recent work from our group demonstrates that two exceptional characteristics of bat biosonar reflect biomechanical constraints of the vocal apparatus.
First, across bat species, there exists a negative correlation between body size and the peak frequency of their echolocation calls; smaller species use higher frequency calls than larger ones. We hypothesized that smaller bats, with smaller mouths, emit higher frequencies to achieve sufficiently directional sonar beams, and that variable directionality is critical for bats. We found that six vespertilionid species (4–21g) produced sonar beams of similar shape and volume, and predict that many bats adjust their acoustic field of view to suit habitat and task. We speculate that sonar beam shape has been an evolutionary constraint on echolocation and best explains the bat size–call frequency correlation.
Second, we discovered that vespertilionid bats use specialized superfast muscles to power rapid call rates.

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12:15 Wednesday 3rd July 2013

A2.4
Nasal sound production in toothed whales

Peter T Madsen (Aarhus University, Denmark)

Toothed whales display a series of remarkable secondary adaptations to a life in water including a highly specialized nasal complex that serve to produce clicks for echolocation and whistles for communication. Both sound types are produced by different actuation of two phonic lip pairs found just beneath the blow hole.
Whistles are formed by pneumatically induced tissue vibrations in the left pair of phonic lips and not by the resonance frequency of the associated air sacs, making the term whistle a functional misnomer. Clicks are also generated through pneumatic actuation, but in this case via a brief opening of the right pair of phonic lips under tension. Air is recycled and as little as 50 µL of air is used to make a single click that may vary over four orders of magnitude in energy and several octaves in frequency depending on driving air pressure and tension of the phonic lips.
The very large hydrostatic pressures during deep dives do not affect click properties, but the number of clicks produced between air recycling events. Whistles on the other hand use more air for production, and their output levels and duration are reduced with depth whereas the frequency is unaltered. Thus, the same sound sources in the toothed whale nasal complex can be pneumatically actuated to generate sound with very different functional properties despite that deep dives offer little support for air-driven sound production.

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12:40 Wednesday 3rd July 2013

A2.5
Vertebrate vocal production and the evolution of human speech

W Tecumseh Fitch (University of Vienna, Austria)

Our understanding of vocal production in vertebrates has undergone a transformation in the past decades, thanks partly to a productive interplay with human voice science. Such theories as the source–filter theory of vocal production and the myoelastic aerodynamic theory of the voice source, originally developed to understand human speech, have been generalized to a wide range of vertebrates (especially mammals and birds). But the exchange has not been one-way: research on non-human animals has also provided surprising insights into the evolution of the human voice. I will use the recent flowering of research on animal formants as an example to illustrate this interchange, and end with some open problems (especially concerning source–tract interactions, and ultrasound production) that deserve focused attention in the future.

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14:00 Wednesday 3rd July 2013

A2.6
Vocal dynamics and sensory feedback in birdsong

Roderick A Suthers (Indiana University, United States)

The production of birdsong requires the precise coordination of muscles at various levels of the vocal system. The fundamental frequency of the vocal source, the syrinx, is determined by the respiratory and syringeal muscles. As this sound passes through the suprasyringeal vocal tract it may be filtered by vocal tract resonance to produce formants.

Although auditory feedback is essential during song learning and in the long-term maintenance of song, the role of real-time sensory feedback during the production of adult song is not well understood. Our experiments with northern cardinals indicate the importance, to song production, of real-time sensory feedback to the syringeal source and the suprasyringeal vocal tract filter.

Both expiratory and syringeal muscles respond by decreasing or increasing, respectively, the amplitude of their electromyogram to small, externally imposed changes in respiratory pressure applied just before or during a song syllable. Both of these responses should tend to stabilize the rate of syringeal airflow during phonation. Neither response is abolished by deafening, indicating it depends on proprioceptive or mechanoreceptive, rather than auditory, feedback.

In other experiments, we have shown that cardinals singing in heliox attempt to minimize the upward shift in formant frequency that accompanies light gas by reducing beak gape, which reduces the resonance frequency of the upper vocal tract. Deaf birds do not reduce their beak gape in heliox, indicating the compensatory gape reduction by hearing birds depends on auditory feedback.

Supported by grant NIH-NINDS/2R01NS029467

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14:45 Wednesday 3rd July 2013

A2.7
Using a low dimensional birdsong model to unveil neural coding in zebra finches

Ana Amador (University of Buenos Aires, Argentina), Yonatan Sanz Perl (University of Buenos Aires, Argentina), Gabriel B Mindlin (University of Buenos Aires, Argentina) and Daniel Margoliash (University of Chicago, United States)

Songbirds are a well studied example of vocal learning that allows us to integrate neural and peripheral recordings with a precisely quantifiable behaviour. Although neural activity in the premotor forebrain nucleus HVC has been related to song acoustics in auditory playback experiments, it remains unresolved whether neural activity is related to song spectral structure during singing. To address this issue, we worked with a minimal physical model for birdsong production, having as an output a synthetic song. Each syllable was coded in terms of parameters related to air sac pressure and tension of the syringeal labia, defining motor 'gestures'. To validate this model, we assessed responses of HVC neurons to song playback in sleeping birds, as HVC neurons exhibit highly selective responses to the bird's own song (BOS). Remarkably, the mathematical model was able to elicit responses strikingly similar to those for BOS, with the same phasic-tonic features. These results demonstrate that a low dimensional model representing an approximation of peripheral mechanics is sufficient to capture behaviourally relevant features of song.

Analysing the HVC neurons responses to playback of each bird's own song, we observed that projection neurons were excited and interneurons were suppressed, with near-zero time lag, at the times of gesture extrema. We confirm these results with HVC recordings in singing birds.

Given that HVC activity occurs with near synchrony to behavioural output, we propose that the activity of HVC neurons represents the sequence of gestures in song as a 'forward' model making predictions on expected behaviour.

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15:10 Wednesday 3rd July 2013

A2.8
Using CT to predict vocal tract resonances in male fallow deer vocalizations: Are groans nasalized?

David Reby (University of Sussex, United Kingdom), Megan T Wyman (University of Sussex, United Kingdom), Roland Frey (Leibniz Institute for Zoo and Wildlife Research, Germany) and Joel Gilbert (Laboratoire d'Acoustique de l'Université du Maine, France)

Males of several species of polygynous deer (including red deer and fallow deer) have a descended larynx, which gives them an unusually long vocal tract (VT). They can also extend their VT by further lowering their larynx during the production of their sexual loud calls. Formant frequencies are lowered as the vocal tract is extended, as predicted when approximating the vocal tract as a uniform quarter wavelength resonator. However, formant frequencies in polygynous deer appear to obey uneven distribution patterns, suggesting that the vocal tract shape is in fact rather complex.

We CT-scanned the artificially extended vocal tract of two adult fallow deer, and measured the cross-sectional area of the supralyngeal vocal tract (nasopharynx, oropharynx, oral and nasal cavities), along the oral and nasal pathways. We used these data to model resonances patterns produced by these VT including the oral pathway, the nasal pathway, or both. We found that the combined oral/nasal VT produced a formant pattern that more closely matches that observed in fallow deer groans. We also found that this configuration enables a better estimation of VT length from formant patterns (explaining why the inclusion of nasal formants led to an overestimation of apparent vocal tract length in previous studies modelling the fallow deer VT as a single uniform tube). This clear indication that the nasal cavity and oral cavity are both simultaneously involved in the vocal production of a nonhuman mammal suggest that the potential for partial nasalization of putative oral loud calls should be carefully considered.

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16:00 Wednesday 3rd July 2013

A2.9

An attractive larynx: Sexual selection on larynx morphology and the biomechanics of producing complex calls in túngara frogs

Nicole M Kime (Edgewood College, United States) and Michael J Ryan (University of Texas at Austin, United States)

The túngara frog, *Physalaemus pustulosus*, is a well-known model for investigating how sexual selection influences the evolution of acoustic communication. All male frogs in the genus *Physalaemus* produce a species-specific 'whine'. Túngara frogs and some populations of its sister species, *P. petersi*, also produce a second complex call component, a 'chuck' or 'squawk' that is attractive to females and thus favoured by sexual selection. Males in species or populations that produce chucks or squawks have a larger fibrous mass extending from their vocal folds than those without. In túngara frogs, surgical ablation of the fibrous mass deprives males of the ability to produce chucks. Sexual selection is thus responsible for the evolution of laryngeal morphology, including the enlarged fibrous mass that supports chuck production. In spite of this evidence for a structure–function correlation, the mechanism by which the fibrous mass interacts with other elements of the vocal system to produce complex calls remains unknown. We have recently been using a lumped element modelling technique called bond graphs to investigate the biomechanics of vocal production in túngara frogs. These models explore the mechanics of the fibrous mass in chuck production and how the production of whines and chucks depends on integrated vocal system morphology. The evolution of the túngara frog vocal system in response to sexual selection likely required correlated changes in many aspects of larynx morphology.

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16:25 Wednesday 3rd July 2013

A2.10

Sound production mechanism in elephant infrasound vocalizations

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The sound production of most mammals can be explained by one of two fundamentally different sound production mechanisms: According to the myoelastic-aerodynamic (MEAD) theory of sound production, the primary sound source is generated by flow-induced self-sustaining oscillations of the vocal folds. In an alternative mechanism, sound is created by active muscle contractions (AMC). Here, a regular pattern of successive EMG bursts (e.g. 20–30 Hz for cat purrs) causes the intrinsic laryngeal muscles to modulate the respiratory airflow. Elephants are the largest land mammals. They produce low-frequency vocalizations in the infrasonic range (fundamentals below 20 Hz). Both AMC and MEAD have been suggested in the literature as sound production mechanisms, but to date no physiologic evidence for either case has been produced. Using high-speed video, acoustic and electroglottographic recordings, we documented flow-induced, self-sustaining oscillations of the vocal folds of an excised elephant larynx (*Loxodonta africana*) at fundamental frequencies below 20 Hz. We also observed a range of nonlinear phenomena, which are directly comparable to those documented in humans and other mammals. Due to the absence of any neural signals in the excised larynx setup, the AMC mechanism can be rejected for elephant infrasound vocal production. Rather, sounds are produced in a manner directly paralleling human speech or song. We conclude that the same physical principles of voice production apply to mammals of various sizes (i.e. bats, humans, elephants), and that the MEAD theory extends across a remarkably wide range of body sizes and vocal frequencies (more than four orders of magnitude).

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16:50 Wednesday 3rd July 2013

A2.11

The songbird syrinx morphome: A three-dimensional, high-resolution, interactive morphological map of the zebra finch (*Taeniopygia guttata*) vocal organ

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Songbirds learn their species-specific vocalizations through imitation learning. The birdsong system is a widely-used experimental animal model for understanding the underlying neural mechanisms responsible for vocal production learning but how neural impulses are translated into the precise motor behaviour of the complex vocal organ (syrinx) to create song is poorly understood. We lack a detailed understanding of syringeal morphology. To fill this gap we combined non-invasive (high-field magnetic resonance imaging, micro-computed tomography) and invasive techniques (histology, micro-dissection) to construct the annotated high-resolution three-dimensional dataset (morphome) of the zebra finch syrinx. We identified and annotated syringeal cartilage, bone and musculature *in situ* in unprecedented detail. We provide interactive three-dimensional models that greatly improve the communication of complex morphological data and our understanding of syringeal function in general.
Our results show that the syringeal skeleton is optimized for low weight driven by physiological constraints on song production. The present refinement of muscle organization and identity elucidates how apposed muscles actuate different syringeal elements. Our dataset allows for more precise predictions about muscle co-activation and synergies and has important implications for muscle activity and stimulation experiments. We also demonstrate how the syrinx can be stabilized during song to reduce mechanical noise and enhance repetitive execution of stereotypic motor patterns. We also identify a cartilaginous structure suited to play a crucial role in the uncoupling of sound frequency and amplitude control, which permits a novel explanation of the evolutionary success of songbirds.

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Poster session –Thursday 4th July 2013

A2.12

Quantifying avian sound production using high-speed kymography and electroglottography

Jeppe H Rasmussen (University of Southern Denmark, Denmark) and Coen PH Elemans (University of Southern Denmark, Denmark)

The size and location of the avian vocal organ, the syrinx, makes it difficult to image tissue movement during sound production in freely singing birds. A novel *in vitro* approach allows us to study the excised syrinx in unprecedented detail. We focus on pigeons as an ideal model species to start understanding the physics and control of sound production. The pigeon syrinx is of relatively simple morphology: it contains only one bilateral pair of vibrating masses, the lateral tympaniform membranes (LTM), and two paired muscles controlling LTM position and tension. We systematically varied bronchial and air sac pressure across and beyond a range of physiological values to investigate the oscillatory behaviour of the excised pigeon syrinx. We imaged LTM movement during controlled air-induced sound production using high-speed video (<10 kHz) and endoscopic kymography (7.2 kHz). The LTMs demonstrate self-sustained oscillations that generate the radiated sound. We observed at least two stable vibrational modes with distinct open–closed quotients. We also tested the applicability of miniaturized electroglottography (EGG) on the excised syrinx. Syringeal opening and thus LTM contact clearly correlates to EGG waveform shape, making it a promising avenue to explore sound production mechanisms and control in freely singing birds.

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Poster session –Thursday 4th July 2013

A2.13

Singing in a jar: The neuromuscular control of the songbird syrinx *in vitro*

Coen PH Elemans (University of Southern Denmark, Denmark)

Song behaviour depends on the integrated action of neural systems for auditory perception, song production, song learning and the processing of social information. Many components of these specialized and interacting neural circuits have been identified but mechanistic insights into their function remain incomplete. To understand the neural basis of birdsong we need a more detailed knowledge of how neural motor patterns are translated into sound in the peripheral sound producing system. The accessibility and small size of the syrinx make it difficult to visualize modulation of syringeal parameters in undisturbed freely singing birds *in vivo* and this experimental setback constraints progress in understanding how neural signals translate into the acoustic output for vocal communication.

Here, I present a novel experimental setup that allows for studying the syrinx in vitro under experimentally controlled conditions. The setup combines independent and accurate control of air pressure and flow downstream (bronchial), upstream (tracheal), and in the air sac system surrounding the syrinx, with synchronized high-speed visualization of labial movement from different orientations. Furthermore, the syrinx and its associated musculature can be kept alive for several hours using micro-perfusion techniques. This addition allows for quantitative study of controlled muscle recruitment on (1) the biomechanical/kinematic effects of the modulation of structural elements and (2) the control of the syringeal pressure parameter space with its associated acoustic modulations. This setup allows for studying basic sound production physiology and opens the way to comparative studies of sound production in birds, mammals and frogs.

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Poster session –Thursday 4th July 2013

A2.14

Personality traits identification based upon hypoxia test and mecano acoustic stimulation in European seabass (*Dicentrarchus labrax*)

David Benhaïm (CNAM–INTECHMER, France), Sébastien Ferrari (IFREMER, France), Romain Theysset (Holar University College, Iceland) and Marie-Laure Bégout (Ifremer, France)

Two divergent behavioural and physiological response patterns to challenges or stimuli have been identified in animals and frequently termed proactive and reactive coping styles. These individual differences may result in suboptimal production and compromised cultivated fish welfare. An approach to overcome these problems could be to sort fish and optimize rearing conditions according to coping styles. Several methodological approaches have been used to characterize fish coping styles including confinement (Silva et al., 2010, Øverli et al., 2004), recovery of feeding motivation in a new environment (Øverli et al., 2007; Martins et al., 2011), exposure to a novel object (Martinset al., 2011), a combination of test (Castanheira et al., 2013) and aggression tests (Øverli et al., 2004) or Mecano Acoustic Stimulation (MAS, Millot et al., 2009). However, most challenges used to discriminate coping styles or personality traits are done in isolated situations and are highly time consuming avoiding possibility for further selection. In seabass, which is a gregarious species, test in isolation could be experienced as a stressful situation and alter individual behaviour. More recently, test in group situation were done with success for screening coping style in fish, including risk-taking tests (Millot et al., 2009; Huntingford et al., 2010) and hypoxia exposure (Laursen et al., 2011, Castanheira et al., 2013). We investigated how a group-based test (hypoxia test) could be predictive of individual responses during a simulated attack predator (MAS). Moreover we highlight the link between hypoxia sensibility and proactive traits and discuss consistency between context in seabass.

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Poster session –Thursday 4th July 2013

A2.15

From audible to ultrasound in stereotypic vocal signals: A phylogenetic exploration in size-dependent trends and taxonomic signature peromyscine mice (Subfamily: Neotominae)

Jacqueline R Miller (Royal Ontario Museum, Canada) and Mark D Engstrom (Royal Ontario Museum, Canada)

Morphology and phylogeny influence the characteristics of vocal signals but a relationship between size and frequency has rarely been tested on a fine scale among closely-related species with a relatively narrow range of size variation. Multiple peromyscine species produce stereotypic vocal signals, and frequency use in these vocalizations varies within and among species and genera. Important to conspecific recognition, such signals are considered the most phylogenetically conserved vocalization categories. We use phylogenetic mapping, PCA, linear regression and independent contrasts to test the hypothesis that body size explains a significant proportion of variation in spectral use among closely related taxa, against an alternative hypothesis that phylogeny constrains the overall character of stereotypic vocalizations. We map ecological variables associated with habitat use/condition onto our phylogeny to explore observations incongruent with either hypotheses.

Phylogenetic categorization of stereotypic signals reflects both genus and tribe. Individual species within genera are distinguishable, but with some overlap in multidimensional acoustic space among the small-bodied species of *Peromyscus*. A moderate but significant negative correlation exists between frequency characters and body size in some variables, a relationship made stronger when controlled for phylogeny. The size–frequency relationship observed reflects specific generic and tribal level trends. Interestingly, there is a strong degree of character similarity between the two genera producing relatively low and constant frequency signals, compared to genera and species that produce frequency-modulated signals.

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Poster session –Thursday 4th July 2013

A2.16

Adaptive prey tracking by echolocating harbour porpoises

Danuta M Wisniewska (Aarhus University, Denmark), Mark Johnson (University of St Andrews, United Kingdom), Kristian Beedholm (Aarhus University, Denmark) and Peter T Madsen (Aarhus University, Denmark)

Echolocating odontocetes produce regular clicks when approaching targets, switching to high-repetition-rate buzzes at short-target ranges but their capability to track prey movements, particularly during buzzes, is limited. Recent studies using multisensor tags on free-ranging beaked whales identified sequences of echoes interpreted as stemming from ensonified prey and accelerometer signatures possibly indicative of feeding. Here we verify this in a controlled environment, and elucidate what echograms may tell us about echolocation during prey capture by applying similar tags to trained harbour porpoises. Tag-synchronized, high-speed, underwater cameras were used to observe details of feeding events. These showed accelerometer signatures occurring when animals engulfed and manipulated prey; likely resulting from rapid movements of gular muscles creating suction by lowering the buccal pressure. Transient drops in clicking rates accompanying the accelerometer signatures suggest that this lowering may impede the pneumatic processes of click production. Echograms enabled tracing of prey ranges during buzzes. Buzz clicking rates followed fast prey movements with delays of ~100 ms, suggesting sub-cortical regulation. We conclude that porpoises can carefully track prey with adjusted click intervals during both approach and buzz phases, but with vocal–motor control in each, mediated through different neural pathways. Seemingly similar rate changes may therefore emerge in three distinct ways: as a passive consequence of the biomechanics of sound production; a reflex; or through deliberate control.

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Poster session –Thursday 4th July 2013

A2.17

Vocal differentiation and development of auditory abilities in a highly soniferous fish

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The development of the vocal repertoire is well documented in birds and mammals but less studied in fish. However, since vocal networks in all vertebrates seem to have evolved from a shared brain area, it is important to understand how vocal abilities differentiate in this less complex taxon. Parallel development of vocal–auditory systems might be important for communication and the control of self-vocalizations.

The Lusitanian toadfish *Halobatrachus didactylus* (Batrachoididae) produces at least five different calls and shows remarkable territoriality in early developmental stages. We aim to describe the development of the vocal repertoire throughout ontogenetic development, and to investigate possible changes with age in peripheral auditory sensitivity and representation of conspecific signals in *H. didactylus.*

Vocal activity was recorded during social interactions in juveniles (g1: 2–5 cm; g2: 5–9 cm SL) and in adults (g3: >19 cm). Evoked potentials were measured from populations of saccular hair cells. Representation of conspecific signals was registered using the AEP technique.

Considerable differences were found in vocal complexity between both juvenile groups. While g1 emitted mostly grunts, g2 exhibited already the full vocal repertoire. Saccular hair cells of the smallest juveniles were significantly less sensitive (~10 dB) than in the other two groups. Auditory responses to conspecific signals indicated a gradual improvement in the representation of various features throughout development, with adult fish presenting a more accurate encoding of the signal.

Our data suggest that the development of the auditory system parallels the development of the vocal system in this highly vocal fish.

Poster session –Thursday 4th July 2013

A3 – MUSCLE–TENDON BIOMECHANICS

Organized by: Manny Azizi (University of California, Irvine) and Polly McGuigan (University Of Bath)

A3.1

Mechanical interactions between triceps surae muscles are mediated by both common elastic and myofascial connections

Huub Maas (VU University Amsterdam, Netherlands)

The triceps surae muscles play an important role during the stance phase of locomotion. This becomes particularly evident following (partial) rupture of the Achilles tendon. It consists of three muscles (m. gastrocnemius medialis and lateralis, and m. soleus) with different independent origins but inserting together as the Achilles tendon in the calcaneus. Even though three fascicles can be distinguished, dissection of each tendon fascicle in rat becomes progressively more difficult towards the calcaneus, suggesting that these fascicles unite in one tendon. Besides this shared distal tendon, the triceps surae are mechanically linked via connective tissues at their shared muscle belly interface (i.e., epimuscular myofascial linkages). Both structures link the action of one muscle to its adjacent synergists.

Via epimuscular linkages, muscle fibre force can be transmitted to the tendon of a neighbouring muscle. Via a common tendon, force exerted by one muscle affects the length at which muscle fibres in the adjacent muscle are contracting. This complex structural arrangement of triceps surae can have substantial consequences for the mechanical effect of each muscle at the joint. We have investigated the effects of muscle connectivity for the transmission of triceps surae muscle forces onto the skeleton in the rat.

Lengthening gastrocnemius proximally, simulating knee movements, causes significant changes in forces exerted at the distal tendon of soleus. Soleus ankle moments assessed at various ankle and knee joint angles indicate mechanical connectivity with gastrocnemius muscle, mediated by effects of myofascial linkages and common elasticity.

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10:30 Thursday 4th July 2013

A3.2

The role of the tibialis anterior muscle and tendon in absorbing energy during walking at different speeds and slopes

Glen A Lichtwark (University of Queensland, Australia), Thomas Halt (University of Queensland, Australia) and Andrew G Cresswell (University of Queensland, Australia)

The human tibialis anterior (TA) muscle has two distinct roles during walking:
(1) concentrically dorsiflex the ankle during swing for foot clearance and placement; and
(2) eccentrically contract during ankle plantar-flexion in the initial foot contact with the ground.

The latter role is interesting because eccentric muscle activity can potentially cause muscle damage unless the elastic tendon undergoes most of the stretch. We hypothesized that the TA tendinous tissue would undertake most of the lengthening during initial foot contact of walking and that this stored energy could be utilized or dissipated during muscle relaxation. TA muscle fascicle and MTU length changes were measured using ultrasound imaging and motion analysis, while muscle activity was recorded with surface electromyography during walking at three speeds (Fr 0.1, 0.25 and 0.4) and three slopes (-15° , 0° and 15°). During walking with no slope, the MTU actively lengthened during the initial contact phase of the foot while fascicles acted isometrically, or even shortened with higher speeds – absorbing / storing energy in the tendinous tissues.

During muscle relaxation, the MTU shortened while the fascicles lengthened, absorbing some, but not all, of the energy stored in the tendon. Slope changed the MTU and fascicle dynamics, however energy was always stored in the tendon during initial contact.

The results suggest that initial energy absorption during foot contact is achieved primarily within the TA tendinous tissue and that some of this energy is returned (and some dissipated), depending on the requirements of the walking task.

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11:10 Thursday 4th July 2013

A3.3

Muscle–tendon interaction during human locomotion with elastic exoskeletons

Gregory S Sawicki (North Carolina State University, United States) and Dominic J Farris (University of Queensland, Australia)

Wearable devices that can 'off-load' biological muscle–tendon units during functional movements are no longer limited to the realm of science fiction. Powered and passive exoskeleton systems have evolved to be lighter, stronger and 'smarter'; but is still unclear how mechanical assistance from these devices influences muscle-level mechanics and energetics during locomotion.

In the Human PoWeR (Physiology of Wearable Robotics) laboratory, we have developed a framework that includes: (1) simple computational models; (2) a novel *in vitro* muscle–tendon workloop paradigm; and (3) *in vivo* measurement techniques based on combining inverse dynamics and ultrasound imaging data to ask: How does an elastic exoskeleton (i.e. exotendon) working in parallel with a compliant biological muscle–tendon unit influence muscle–tendon interaction dynamics?

Through a number of examples, I will highlight the fact that exoskeletons designed to assist a lower-limb joint may have unintended consequences when examining changes in underlying muscle dynamics. As one example, I will present results from *in vivo* ultrasound measurements that indicate elastic ankle exoskeletons reduce force but not mechanical work of the soleus muscle during spring loaded vertical hopping. In another example, I will present modelling results that indicate the potential for increased muscle injury risk during locomotion with assistive exotendons. Finally, I will discuss approaches to optimize exotendon properties to improve muscle-level mechanics and energetics.

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11:40 Thursday 4th July 2013

A3.4

How can the muscle be organized to accomplish tendon displacement at the calcaneus that are similar to the muscle fibre length?

Ryuta Kinugasa (Kanagawa University RIKEN, Japan)

It is generally agreed that most soleus muscle fibres are 3–4 cm in length. However, ankle rotation movements suggest a similar magnitude for the excursion of the Achilles tendon (~3 cm) in ankle rotation. How can the muscle be organized to accomplish tendon displacement at the calcaneus that are similar to the muscle fibre length?
In-vivo measurements of muscle shortening during ankle movements show that the distance moved by the calcaneus exceeds the shortening of the muscle fibres. Thus, mechanisms must exist between the muscle fibre and calcaneus which amplify the muscle fibre length changes. One such system appears to be the internal mechanics of the muscle that a pennate fibre arrangement between aponeuroses that remain a constant distance apart during a contraction would result in a movement of the aponeurosis parallel to the long axis of the muscle that exceeded the shortening of the muscle fibre. The second system operating at the ankle and observed curvature of the Achilles tendon under loading indicates the presence of a mechanical constraint close to the ankle which limits posterior movement of the tendon as the ankle rotates. Such a constraint suggests that it would modify the relationship between muscle shortening and ankle rotation, adding more amplification to the translation of muscle fibre shortening to ankle rotation.
As best can be done with the present data available, I will attempt to explain how the human soleus muscle–tendon complex generates movements at the calcaneus that are almost equal to the length of its muscle fibres.

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12:10 Thursday 4th July 2013

A3.5

Tendons may be a drag during drag-based swimming

Christopher T Richards (Harvard University, United States), Angela RV Rivera (Harvard University, United States) and Udit Choudhury (Harvard University, United States)

Based on recent theory and experimental evidence, most physiologists now expect that spring-like tendons enhance locomotor performance, especially for ballistic behaviours such as frog jumps. However despite the dramatic skeletal transformations driving the evolution of frog jumping, many species are also impressive swimmers either obligatorily or facultatively. Are swimming kicks simply 'underwater jumps'? Do the muscle–tendon (MT) properties that confer jumping ability also benefit swimming? Using both numerical simulation and 'musculo-robotic' instruments based on the anatomy and muscle properties of the *Xenopus laevis* ankle joint, we explored three alternative hypotheses: the optimal MT properties for jumping either: (1) enhance swimming; (2) attenuate swimming performance; or (3) have no impact on swimming ability.
Simulation and bio-robotic trails were performed in two flow regimes: rotating with forward translation (T_1) or in still water without translation (T_0). Additionally, the foot moment of inertia, drag coefficient, tendon stiffness and mechanical advantage were manipulated in search of 'optimal' anatomical and physiological properties for producing MT power. Preliminary analysis suggests that compliant tendons may attenuate MT power by as much as ~80% in T_0 trials, however, do not alter net MT power output in T_1 trails.
Thus our findings do not predict performance benefits of elastic recoil for drag-based fluid loads. At best, compliant tendons appear not to influence net power output, but also can greatly attenuate MT power, depending on the translating flow regime.

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12:25 Thursday 4th July 2013

A3.6

Biaxial material properties of fascia lata and the functional implications for movement

Carolyn M Eng (Harvard University, United States), Francesco Q Pancheri (Tufts University, United States), Daniel E Lieberman (Harvard University, United States), Andrew A Biewener (Harvard University, United States) and A Luis Dorfmann (Tufts University, United States)

Like tendon, fascia is composed primarily of collagen and connects muscles to bones. Previous studies have shown that fascia may play a similar role to tendon in locomotion by providing limb stability, transmitting muscle force, and storing and recovering elastic energy. While tendon is rope-like, fascia has a sheet-like structure broadly attaching to muscles and bones at multiple sites. Therefore, fascia is undoubtedly exposed to different states of biaxial strain and its functional potential cannot be captured with a simple uniaxial testing protocol.
Planar biaxial tests with strain control were performed on goat fascia lata (FL). Because the two collagen layers in the FL are oriented approximately perpendicular to each other, we performed biaxial tests on longitudinal and transversely oriented samples in each goat. Samples were cycled to multiple strain levels while the non-cycling direction was held constant at 0% and 3% strain. Structural differences in the collagen layers were examined using histology. Results show that FL stiffness and hysteresis are greater in the longitudinal *versus* transverse direction and the increased stiffness in the longitudinal layer is likely due to its greater thickness compared to the transverse layer.
Our results have implications for fascia function. Differences in material properties between the longitudinal and transverse orientations enhance the ability of the longitudinal FL to transmit force, store energy, or stabilize the limb. Additionally, the relative compliance of the transverse fibres may allow expansion of underlying muscles during contractions.

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12:40 Thursday 4th July 2013

A3.7

Muscles with split personalities: Can one muscle change its function during a single task?

Taylor Dick (Simon Fraser University, Canada) and James M Wakeling (Simon Fraser University, Canada)

Muscles have a variety of functions during locomotion and can act as motors, brakes, springs, and struts. The role of the gastrocnemii during cycling is still controversial. Some suggest they act like struts and transfer power while others state they function like motors to propel the crank. We aimed to evaluate the power and timing shifts in the medial gastrocnemius (MG) during cycling using the workloop technique.
We tracked the MG distal muscle–tendon junction and kinematics for six subjects. AT forces during cycling were calculated from measured changes in tendon length. Workloops were generated for each cycling condition and power output calculated. We used cross correlation to evaluate the timing (phase shift) between belly length and AT force.
As bike power increased with a fixed crank torque of 7.2 Nm, there was a significant increase in muscle power output and phase shift. This suggests that the MG plays a key role as a motor during high speed tasks, possibly a function of its high proportion of fast muscle fibres. As crank power increases at a fixed cadence the MG acts like a strut to transfer power from the large and powerful quadriceps muscles. These changes in function may be driven by changes in timing between the muscle length and its force development. For the first time, we have used *in-vivo* measures of muscle belly length and AT force to display that the MG is in fact a schizophrenic muscle and its role changes with the mechanical demands of the task.

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Poster session – Thursday 4th July 2013

A3.8

The effect of tendon compliance on swimming performance: Is it mediated by force-length effects and starting length?

Christopher T Richards (Rowland Institute at Harvard University, United States) and Angela RV Rivera (Rowland Institute at Harvard University, United States)

Tendons can enhance performance by storing elastic energy produced by the muscle, then releasing it rapidly during recoil, assisting the muscle to accelerate the load. Under certain conditions, tendon dynamics cause 'power amplification', where peak muscle–tendon power exceeds theoretical limits of contractile element (CE) power. While power amplification is likely in jumping, its role during swimming is less known. Recent models of MT dynamics during hydrodynamic loading suggest that multiple morphological configurations may maximize MT power, sometimes causing power amplification. However, as this work was performed in still water, it is unclear whether compliant tendons enhance or reduce swimming speed. To address this, we used a forward translating bio-robotic foot actuated by motors simulating *Xenopus laevis* muscle dynamics. We simulated muscle contractions at tendon stiffness values ranging from 750 to 30,000 N/m allowing us to relate MT and whole body swimming performance. To determine the effect of force-length (FL) properties, FL effects were either excluded (FL-) or included (FL+) in our muscle model, in addition to varying muscle starting length. For FL-, we found power amplification for all but the stiffest tendon. For FL+, we found peak power to be dependent on starting length, particularly for intermediate stiffness values. Preliminary analysis suggests that although FL- trials showed no effect of tendon compliance on swimming speed, FL+ trials suggest that highly compliant tendons may reduce swimming speed.

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Poster session – Thursday 4th July 2013

A3.9

Can bat wing muscles stretch their tendon to enable force control of joint movement?

Nicolai Konow (Brown University, United States), Rhea Von Busse (Brown University, United States), Thomas J Roberts (Brown University, United States) and Sharon M Swartz (Brown University, United States)

When muscles contract, they may control joint position directly, but can also stretch series elastic tendon, which potentially leads to force control of joint movement. Hallmarks of force control for instance include the shortening of a monoarticular extensor muscle, while the joint it crosses remains stationary or is flexed. Studies of diverse species have revealed a widespread occurrence and important roles of force control in terrestrial locomotion. However, we know little about its involvement in locomotion through fluids, for which modelling studies have suggested potential disadvantages from series elastic compliance. It also remains unclear if limb muscles of small mammals can generate sufficient force to stretch their relatively stiff and thick tendons.

To probe these questions, we studied *in vivo* function of wing muscle–tendon units in the bat *Carollia perspicillata* during take-off and climbing flight. Wing joint kinematics were measured using biplanar fluoroscopy, muscle strain using fluoromicrometry, and muscle activity using electromyography. We found several indications of tendon being stretched. For instance, during early downstroke, the acromial head of biceps frequently shortened while the elbow joint remained relatively extended. During upstroke, the monoarticular head of triceps was activated earlier than seen during steady flight, and shortened against elbow flexion. These findings indicate that the action of muscles powering animal movements through fluids may be influenced by series elasticity, and that at least some limb tendons in small mammals can be stretched by muscular and aerodynamic forces, enabling force control of joint movement.

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Poster session – Thursday 4th July 2013

A3.10

Frequency response of a biological muscle–tendon unit experiencing dynamic inertial load

Benjamin D Robertson (UNCNC State Biomedical Engineering, United States) and Gregory S Sawicki (UNCNC State Biomedical Engineering, United States)

A biological muscle–tendon unit (MTU) can significantly reduce energy costs with little effect on overall power output by minimizing muscular length change, and maximizing energy storage and return in series elastic tissues. This concept of 'elastic tuning' is a classic principle of controls engineering, and is based around understanding system frequency response to dynamic loading.

To characterize the frequency response of biological muscle–tendon systems, we developed a bio-robotic experimental framework using plantaris–Achilles tendon MTU from *Rana catesbeiana* with two key differences from classical muscle workloop studies. First, series tendons are left intact and muscles are instrumented with sonomicrometry to decouple component mechanics. Second, system dynamics rely on interaction of simulated inertial environment and muscle–tendon mechanics, not imposed trajectories.

We manipulated simulated inertial parameters to give our muscle–tendon preparation a passive resonant frequency of 2.4 Hz, and drove it with 10% duty for six cycles at frequencies ranging from 1.8–3.2 Hz. Stable, cyclic mechanics were observed in all conditions after two to three cycles of stimulation. The greatest peak force/average positive power generated by the MTU (0.81 P/P_0, 0.028 mW) and series tendon (0.033 mW) occurred at a driving frequency of 2.6 Hz, just above passive resonance. In general, tendon average positive power exceeded that of muscle in all conditions, and exceeded MTU average positive power for frequencies ≥2.4 Hz. The muscle itself was a net power generator for frequencies ≤2.6 Hz, produced net zero power at 2.8 Hz, and was a net energy dissipater for frequencies >2.8 Hz.

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Poster session – Thursday 4th July 2013

A3.11

Mechanisms governing muscle shape change during locomotor activities

Nicholas J Gidmark (Brown University, United States), Nicolai Konow (Brown University, United States) and Thomas J Roberts (Brown University, United States)

The change in shape of muscles as they contract significantly impacts contraction force and velocity. Within a single muscle, shape change can be variable: for example, muscles bulge in different directions depending on the force of a contraction. Although shape change has been characterized in several muscles, its underlying mechanisms remain unclear. To determine if the aponeurotic (tendon) sheets of pennate muscles constrain shape changes *in vivo*, we used radiopaque markers implanted along muscle fascicles, at the boundaries of the muscle belly, and within the aponeurosis of turkey lateral gastrocnemius muscles. We triangulated the three-dimensional position of these markers using high-speed biplanar fluoroscopy during jumping and landing to measure instantaneous muscle length, width, thickness, and fascicle pennation angle. These measurements let us compare contractions involving similar forces but contrasting fascicle length trajectories: muscles shorten during jumping and mostly lengthen during landing. We observed qualitatively similar changes in muscle and aponeurosis widths across behaviours. However, the magnitude of aponeurosis strain and change in muscle width can be very different during high-force activities, suggesting that at least some muscle bulging occurs independent of aponeurosis strain. Our results also show that changes in muscle width are not simply a function of muscle force, suggesting that the simplest model of ECM-mediated shape change may not be sufficient to explain variations in muscle bulging.

Supported by NIH grant AR055295.

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Poster session – Thursday 4th July 2013

A4 – MECHANISMS AND FUNCTIONS OF INTRASPECIFIC VARIATION: FROM GENES TO BEHAVIOUR

Organized by: Lynne Sneddon (University of Chester) and Mark Briffa (University of Plymouth)

A4.1

The genomics of personality in a small passerine bird, the great tit

Kees Van Oers (Netherlands Institute of Ecology (NIOO-KNAW), Netherlands)

Animals within populations consistently differ in the way they cope with environmental challenges. This phenomenon is often referred to as animal personality. Personality differences have been shown to be wide-spread and to influence fitness in natural populations. Quantitative genetic variation underlying personality differences has been demonstrated in studies both on wild as well as captive populations. Until recently it was impossible to connect this variation to genome-wide molecular genetic variation. Such a connection is essential to identify genes responsible for phenotypic variation and to study the way these genes interact with the environment in which they are expressed, in order to describe or predict micro-evolutionary processes. Here I show the first results of the genomic characterization of the great tit and its associations with personality.

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10:30 Thursday 4th July 2013

A4.2

Aggressiveness, dominance and the genetics of social competition

Alastair J Wilson (University of Exeter, United Kingdom)

The widespread finding of personality variation in animal populations, has led to considerable interest in understanding both its causes and consequences. One hypothesis is that personality traits such as boldness and aggressiveness will contribute to an individual's competitive ability. If so then under competitive conditions, personality differences will generate variation in resource acquisition, which in turn should increase variation in resource-dependent traits such as growth or reproduction. However, while the success of any individual in a competition will depend directly on its own phenotype (and so potentially genotype) it must also depend indirectly on the phenotypes of its competitors. Using experimental studies of aggressiveness and dyadic contest behaviour in mice and fish we show that these indirect effects on contest outcome can be identified, influence life history variation, and can be heritable. Recently developed evolutionary theory highlights the fact that where indirect effects have a genetic basis, they are likely to play a major role in shaping the evolution of personality as well as life history traits that depend on competitive outcomes. Our empirical results are discussed in this context.

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10:50 Thursday 4th July 2013

A4.3

The relationship between *drd4* polymorphism, corticosterone and behavioural syndromes in the collared flycatcher: Is there a proximate constraint on consistency?

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Consistent between-individual variation in behaviour can be caused by certain physiological constraints, and pleiotropic hormones or genes that govern multiple behaviours are hypothesized to drive behavioural syndromes. Identifying such a shared proximate link is important, because it would imply that selection conserves behaviours mechanistically coupled so that they cannot evolve independently.
To investigate the physiological and genetic background of behavioural consistency in male collared flycatcher (*Ficedula albicollis*) in a long-term project starting from 2007, we monitored three behaviours (novelty avoidance, aggression and risk taking) that are repeatable across trials and form a behavioural syndrome, and related them to estimates of physiological stress and genetic polymorphism.
Concentration of metabolites of corticosterone (the most important stress hormone) in the droppings was not related to behavioural scores along the shy/bold personality spectrum. In a quest for genotype–personality associations, we screened variations in the dopamine receptor D4 gene (*drd4*, known as novelty-seeking gene). Out of the two *F. albicollis drd4* gene polymorphisms examined, a synonymous single nucleotide polymorphism (SNP764) in a coding region was significantly related to risk taking, but not to the other behaviours. Therefore, we did not find correlative evidence for the pleiotropic effects of hormones or genes mediating behavioural syndromes. Furthermore, we also analysed between-year variations in behavioural correlations, which also suggested a weak role for a common proximate constraint on behaviours that would affect the evolution of behavioural syndromes.

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11:05 Thursday 4th July 2013

A4.4
Behaviour, physiology and carotenoid pigmentation in Arctic charr

Tobias Backström (Swedish University of Agricultural Sciences, Sweden)

Stress responsiveness differs between individuals and is part of the definition of stress coping styles. In salmonid fish, stress responsiveness and stress coping styles has been shown to be connected to the melanin-based pigmentation. However, the Arctic charr (*Salvelinus alpinus*) also have carotenoid-based pigmentation. To elucidate if stress coping style and carotenoid-based pigmentation are connected, an experiment evaluating behaviour, stress response, and carotenoid-based pigmentation was performed. The behaviour during an exploration task and the stress response to a confinement stress of Arctic charr was evaluated.
The behaviour of individuals during 90 minutes of exploration was classified into high and low activity individuals. The high activity individuals had higher plasma cortisol levels following stress compared to low activity individuals. This indicates that high and low activity individuals are corresponding to reactive and proactive stress coping style respectively. Further, a pigmentation analysis showed that high activity individuals had a higher number of carotenoid spots per area than low activity individuals.
Thus, carotenoid pigmentation, as melanin pigmentation in other salmonids, could be linked to stress coping style in Arctic charr.

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11:20 Thursday 4th July 2013

A4.5
Know your enemy: Links between metabolic traits and aggression in a tropical damselfish

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Intraspecific competition for resources and territories are common throughout the animal kingdom. An interesting example is the juvenile stage of many coral reef fishes, during which individuals can be extremely territorial as they settle on coral reef habitats. Mortality at this early life-stage is high, and there is strong competition among conspecifics for territories with the lowest risk of predation. Factors that determine which individuals are able to out-compete others to obtain suitable territory are not well understood but are perhaps linked to physiological traits such as metabolic demand or the scope for aerobic performance.
We investigated this link by staging competitions for coral reef habitats between size-matched pairs of juvenile ambon damselfish (n=36 pairs). For each individual, routine and maximal oxygen consumption rates were measured to calculate aerobic scope both before and after each contest. The probability of winning a territorial dispute was analysed in relation to these physiological measurements. The metabolic cost of aggression was also assessed by visually exposing individuals to each other while each was housed in separate respirometry chambers.
Interestingly, fish showed a greater metabolic response when visually exposed to an individual with which they had already competed a few hours prior, compared to when exposed to a total stranger. Links between aggressive behaviours and physiological traits suggest that these characteristics may be important in determining which individuals obtain the best habitats during settlement and, therefore, which individuals survive through this dangerous ecological transition.

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11:35 Thursday 4th July 2013

A4.6
Do androids dream of eclectic seals? Testing for pinniped personalities in wild populations

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Consistent individual differences (CIDs) in behaviour, indicative of behavioural types or personalities, have been shown in taxa ranging from Cnidaria to Mammalia. Despite numerous theoretical explanations there is limited empirical evidence for selective mechanisms that maintain such variation within natural populations. We examined behavioural types and fitness proxies in wild, breeding, female grey seals using a remote-controlled vehicle to deliver a novel auditory stimulus to elicit changes in pup-checking behaviour. Mothers exhibited highly repeatable individual pup-checking rates within and across breeding seasons in response to this mild disturbance. Observations of undisturbed mothers also revealed CIDs in pup-checking behaviour. There was no correlation between an individual's undisturbed and disturbed pup-checking rates, indicating plasticity across situations. The extent to which individuals changed pup-checking rates from undisturbed to disturbed conditions revealed both proactive females, who maintained a similar rate throughout, and reactive females, who increased pup-checking markedly in response to disturbance. Variation in maternal expenditure was greater among more reactive mothers. Consequently pups of more reactive mothers had more varied growth rates centred around the long-term population mean. These patterns were unrelated to a mother's prior experience, degree of inter-annual site fidelity, physical characteristics of their pupping habitat, pup sex or pup activity. These findings suggest variation in behavioural types in this wild population may be maintained by spatial and temporal environmental variation combined with limits to phenotype-environment matching. We discuss the value of observational and experimental approaches to identifying CIDs in natural populations.

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11:50 Thursday 4th July 2013

A4.7
Interindividual variation in urinary amino acids in the Mozambique tilapia: A signal of dominance or individuality?

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The urine of male tilapia (*Oreochromis mossambicus*) contains a pheromone which is – at least in part – composed of a steroid glucuronide. However, the polar fraction (not containing steroids) also contains potent odorants, the identity and relevance of which are unknown. As amino acids are polar, and potent odorants for fish, we measured urinary amino acid concentrations (gas-chromatography/mass-spectrometry) and assessed their contribution to the odour of male urine (electro-olfactogram) – do amino acids form part of the 'dominance pheromone' in tilapia? Urine was taken from males of different social status (dominant, intermediate and subordinate) and passed through C^{18} solid-phase extraction columns. The polar fraction contained highly variable levels (up to 17 mM) of L-arginine, L-glutamate and L-phenylalanine; the concentration of L-arginine (but not L-glutamate or L-phenylalanine) was positively correlated with the donor's social rank. In addition, comparison of olfactory responses to an artificial mixture of amino acids, based on the concentrations in a pool of urine from dominant males, showed that these three constitute most – but not all – of the olfactory potency of the polar fraction. These results show that: i) urinary amino acids constitute part of the olfactory potency of male urine; ii) levels of one, but not others, are correlated to social status; and iii) odorants other than amino acids are present in the filtrate. We suggest that amino acids contribute to a urinary 'signature' odour; this could be important in aggressive male-male interactions, where urine has previously been shown to have a modulatory role.

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12:05 Thursday 4th July 2013

A4.8
How do aggressive fish win fights? An insight from transcriptomics

Angela M Sims (University of Liverpool, United Kingdom), Lynne U Sneddon (University of Chester, United Kingdom), Tom G Pottinger (CEH Lancaster, United Kingdom) and Phillip C Watts (University of Liverpool, United Kingdom)

Aggressive behaviour can be beneficial, for example by allowing individuals to acquire territories and other limited resources. In juvenile salmonids, aggressive behaviour carries energetic costs, such that aggressive individuals tend to grow at slower rates than less aggressive individuals. To understand the molecular mechanisms underlying aggressiveness, rainbow trout, *Oncorhynchus mykiss*, were assessed for aggressive behaviour by dyadic contests. Aggressive fish were those that performed more aggressive acts during a contest and less aggressive performed fewer.
Using next generation sequencing, the transcriptomes of each behavioural type were characterized. Genes previously implicated in aggressive behaviour in vertebrates, such as *POMC*, were differentially expressed. In addition, a high proportion of differentially expressed genes were associated with metabolism, where aggressive animals up-regulated more genes associated with oxidative stress and with oxygen binding, whereas less aggressive trout expressed genes associated with increased respiration.
These findings indicate the mechanisms by which aggressive and less aggressive rainbow trout differentially allocate resources during an interaction and may demonstrate an underlying propensity for competitive ability. More broadly, these results show the utility of using next generation sequencing to understand the networks of genes involved in behaviour.

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12:20 Thursday 4th July 2013

A4.9
Enhancing fish welfare by studying behaviour: How can personality traits reveal adaptation potential?

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In the context of aquaculture expansion and a growing awareness of fish welfare, European projects were launched to support aquaculture development. Among them, COPEWELL (FP7) aims at developing a new integrative framework for the study of fish welfare based on the concepts of allostasis, appraisal and coping styles. It has been clearly identified that animals differ in their behavioural responses to stressful situations and can be clustered in two main categories: proactive and reactive individuals. Proactive tend to actively avoid or manipulate stressful stimuli and have a 'fight or flight' response, as opposed to reactive ones which display a 'freeze and hide' reaction. Studies on animal personality highlight an adaptive value of such individual variability.
We investigate how this adaptive value could be used to enhance fish welfare. In aquaculture environment, where stressful situation are often unavoidable, it appears likely that one type of response could be better suited. To characterize personalities in fish, young sea bass (*Dicentrarchus labrax*) were subjected to both individual (feeding recovery) and group based tests (hypoxia tolerance and risk taking), and physiological sampling was done to link behavioural responses to individual stress physiology. Individual based tests showed a relationship between escape response and cortisol level. Group based tests showed consistent behaviour in hypoxia tolerance, risk-taking and activity.
Results of the different tests will be discussed in relation to their value for both understanding of the underpinning mechanisms involved in variation in individual coping styles and measuring fish adaptation ability.

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12:35 Thursday 4th July 2013

A4.10
Impacts of environmental contaminants on personality traits: Implications for mechanisms and function

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Where differences in behaviour between individuals are stable across a range of contexts, we refer to this variation as 'personality'. Altering both environmental context and physiology, chemical contaminants are predicted to shape the expression of personality traits.
As they are designed to have biological effects at low concentrations, pharmaceuticals, released in wastewater and sewage sludge, are interesting and potentially potent environmental contaminants. Antidepressants, for example, modulate a range of behaviours in humans including stress responsiveness and behavioural flexibility, providing an ecologically relevant and insightful paradigm for studying animal personality. We investigated the effects of environmentally relevant concentrations of fluoxetine (Prozac™) on wild caught starlings (*Sturnus vulgaris*).
Within individuals some, but not all behaviours were repeatable at baseline, so constituted personality traits, and were inter-correlated, indicating a behavioural syndrome in starlings. After 16 weeks of treatment, repeatability of behavioural traits remained unaltered but inter-trait correlations differed between controls and fluoxetine-treated birds. Finally, we found treatment differences in behavioural and physiological consistency.
Environmental exposure to selective serotonin re-uptake inhibitor (SSRIs) and other psychoactive drugs are predicted to alter important behaviours related to fitness such as predator avoidance, exploration and foraging performance. As fluoxetine is a SSRI, our results suggest that, as in other taxa, individual variation in the serotonergic system may explain inter-individual variation in some personality traits in birds

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12:50 Thursday 4th July 2013

A4.11
Characterizing behavioural 'traits': A conceptual and statistical framework

Niels J Dingemanse (Max Planck Institute for Ornithology, Germany) and Yimen G Araya-Ajoy (Max Planck Institute for Ornithology, Germany)

Behavioural ecologists have developed pointed adaptive explanations for variation in behavioural traits. However, this field lacks objective methods for determining which behavioural expressions quantify the 'traits' that correspond to those for which adaptive theory has been developed. To bridge this gap, we propose a conceptual and analytical framework that is based on evolutionary principles and comes with testable predictions about the structure of behaviour(al correlations) within ('plasticity') and between('personality') individuals and environments. Central to this concept is the notion that behavioural traits explicitly represent latent variables that enable suites of expressed behavioural elements to execute a specific function.
We illustrate our framework by analysing data of four agonistic behaviours expressed during 1275 agonistic encounters collected from 371 wild males great tits (*Parus major*) during territorial intrusions; we detail how character state approaches combined with multivariate mixed-effect models and structural equation modelling can be used to statistically evaluate whether these behaviours were expressions of the behavioural trait 'aggressiveness'.

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13:45 Thursday 4th July 2013

A4.12
The role of mothers and others in intraspecific variation

Katherine A Sloman (University of the West of Scotland, United Kingdom)

The effect of maternal stress and conspecific presence during development were investigated for the potential to shape phenotype and generate intraspecific variation within populations. Studies in oviparous brown trout and viviparous guppies demonstrated that elevated concentrations of maternal glucocorticoids increase aggressiveness of offspring and appear to reduce learning ability. Maternal stressors such as mild as alterations in routine husbandry practice were significant enough to alter the behavioural phenotype of offspring.
In rainbow trout and zebrafish, social isolation during development reduced metabolic rate and aggression and increased activity within two behavioural tests of anxiety. Whether these changes in behavioural phenotype are transferred to subsequent generations is currently being investigated.

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14:05 Thursday 4th July 2013

A4.13
Environmental effects on intraspecific variation in the metabolism of fish

Tommy Norin (Aarhus University, Denmark), Hans Malte (Aarhus University, Denmark) and Timothy D Clark (Australian Institute of Marine Science, Australia)

Intraspecific variation in animal behaviour and physiology has been shown to persist over time (i.e., the variation is repeatable) and to influence important fitness-related traits. Using examples from two species of fishes we show how variation in the external environment affects intraspecific variation in standard metabolic rate (SMR), maximum metabolic rate (MMR) and aerobic scope (AS).
In brown trout, a period of moderate food availability was found to affect the SMR hierarchy that existed within the group, such that repeatability decreased over time. This was likely caused by a failure to maintain the advantage of a high-SMR phenotype when food was scarce, as high SMR fish grew less than low-SMR conspecifics.
In contrast, when barramundi were fed to satiation, high growth rates were obtained by high-SMR fish due to higher food intake, despite variability in other environmental factors (salinity, temperature, oxygen). When experimentally changing salinity, temperature and dissolved oxygen, SMR showed a high degree of consistency within the group of barramundi, whereas the MMR and AS hierarchy that existed under initial acclimation conditions were interrupted but later rebuilt as the fish were continually exposed to different environments.
These results suggest that the response of aerobic performance to environmental variability depends on prior experience, and that this response is not necessarily the same for SMR and MMR. In addition, the absolute degree of intraspecific variation in metabolic rate was reduced as the environment was changed, highlighting the importance of such considerations when evaluating the impact of environmental change on species performance.

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14:25 Thursday 4th July 2013

A4.14
How does risk affect behaviour? Multilevel analyses of plasticity, personality and predictability in hermit crabs

Mark Briffa (Plymouth University, United Kingdom)

When animals are observed on multiple occasions, consistent between-individual differences in behaviour, often referred to as animal personality, may be observed. However, this does not mean that the behaviour of a given individual is readily predictable. While some individuals show low levels of variation around their behavioural mean, others show high levels of variation, and there may be significant between-individual differences in this intraindividual variation (IIV) in behaviour. While it has been suggested that IIV might reduce susceptibility to predators, little is known about the functions or causation of IIV. Here we investigate the effects of temperature and predator cues on the startle response duration of hermit crabs, *Pagurus bernhardus*.
For poikilothermic animals, temperature has a direct and multiplicative influence on metabolic rate, which in turn is expected to influence behaviour due to its effect of increased energy requirements. Under both increased temperature and in the presence of predator cues hermit crabs showed elevated IIV. Thus, predictability in behaviour appears to vary with environmental variables that influence the level of risk that individuals face.

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14:40 Thursday 4th July 2013

A4.15
A conceptual framework focusing on feedbacks connecting animal personalities to underlying mechanisms

Andrew Sih (University of California at Davis, United States) and Barney Luttbeg (Oklahoma State University, United States)

Recent models have sought to explain why animals exhibit behavioural consistency across time and/or across contexts (i.e., why animals have a personality). We seek to explain not only why animals have a personality, but more broadly, to explain variation in the phenomenon. When do we expect animals to exhibit long-term, consistent differences in behavioural type versus a lack of consistent differences? To address this variation, we present a general framework based on the relative importance of positive *versus* negative feedbacks between state variables (e.g., assets, condition, information state, morphology, physiology, social status or role, parasite load) and behaviour. Positive feedbacks tend to cause divergence into different behavioural types while negative feedbacks tend to erode consistent differences in behaviour. We illustrate the interplay of positive *versus* negative feedbacks with specific models. As time permits, we will then discuss predictions on major issues about personalities including:
(1) When should early experiences have major effects on an individual's later behavioural type?
(2) When do we expect animals with higher assets to be cautious and unaggressive versus bolder and more aggressive?
(3) When do we expect populations to exhibit a biomodal distribution of behavioural types as opposed to a continuous range of variation in behavioural types?

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15:45 Thursday 4th July 2013

A4.16

The evolution of personalities in frequency-dependent games: An experimental approach

Morgan David (University of Exeter, United Kingdom)

Explaining the evolution of personalities requires identifying both the factors promoting among-individual behavioural differences and those favouring individual consistency. Whereas much effort has been devoted to understand the maintenance of among-individual variation within populations, the question of why and when within-individual consistency may be promoted remains unanswered.
Recent theoretical models have suggested that, when foraging in groups, individuals may display a certain degree of consistency in the use of producer and scrounger foraging tactics. In this situation the frequency-dependence of expected payoffs is thought of preventing the flexible strategy to reach the ESS. Here I provide an experimental test of two such recent models using zebra finches (*Taeniopygia guttata*), ground-feeding passerine birds, foraging in flocks. I thus have investigated the effects of sequential access to resources and environmental heterogeneity on within-individual consistency in the use of producer and scrounger tactics.
In support of theoretical predictions, I found that a sequential access to resources had strong effects on within-individual consistency. Furthermore, environmental heterogeneity was shown to promote higher levels of flexibility in females whereas males remained more consistent overall.
These results shed light on how individual consistency can evolve within populations, and may also have implications for the evolution of responsiveness or social information use in animals.

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16:00 Thursday 4th July 2013

A4.17

Boldness consistency across contexts in male two-spotted gobies

Carin Magnhagen (Swedish University of Agricultural Sciences, Sweden), Sebastian Wacker (Norwegian University of Science and Technology, Norway), Elisabet Forsgren (Norwegian Institute for Nature Research (NINA), Norway), Lise Cats Myhre (Norwegian University of Science and Technology, Norway), Elisabeth Espy (Norwegian University of Science and Technology, Norway) and Trond Amundsen (Norwegian University of Science and Technology, Norway)

In recent studies of animal personality, much work has focused on how various dimensions of personality are related. Less attention has been paid to whether certain personality traits show up across a variety of situations or contexts. In this study we test whether individuals that consistently behave boldly in one personality assay would also be bold in a range of other test situations. Our model organism was the two-spotted goby, a small marine fish.
Two-spotted goby males were tested during the breeding season for consistency in risk-taking behaviour, within and between personality tests. We used two standard personality assays (the open field test and the emergence test), and two simple assays related to threat response. Repeated runs of each of the tests were highly correlated, and we found significant correlations between all four assays. Thus, we document a between-context consistency in risk-taking behaviour, with a comprehensive set of boldness tests. Furthermore, we found that the goby males studied during the middle of the breeding season were bolder than those studied at the end of the season. This fits nicely with the sexual dynamics of this model species, since male two-spotted gobies face strongly decreasing male-male competition as the season progresses.
The benefits of a bold behaviour may thus differ over the season, and the seasonal effect on boldness might be explained either by individual plasticity or different life history strategies.

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16:15 Thursday 4th July 2013

A4.18

Stability of behavioural syndrome and the underlying mechanisms: What can be learned from the study of parasitic manipulation?

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There is an ongoing interest in documenting how broad and stable behavioural syndromes are, as well as their underlying mechanisms. The role of parasites in the evolution of behavioural syndromes still remains unexplored. Yet, parasite-induced phenotypic alterations can potentially generate or uncouple behavioural syndromes for at least two reasons: first because several related behavioural dimensions may be altered; and second because some of these changes may result from selection acting to promote parasites' transmission at the expense of hosts' fitness.
Here, we review the insights gained from taking a behavioural syndrome approach to the study of parasitic manipulation, and reciprocally, what parasitic manipulation can tell us on the stability of behavioural syndrome and its underlying mechanisms. More specifically, we question the nature of the changes induced by manipulative parasites on risk-sensitive traits and on trait plasticity *versus* consistency. We also propose that changes in key neuromodulatory systems could explain both behavioural syndrome structure and parasite-induced multidimensional changes.
To illustrate our integrative approach, we present original data on the structure of a behavioural syndrome in the freshwater crustacean *Gammarus pulex*, its modification following infection with an acanthocephalan parasite, and the possible role of the serotonergic system in inducing these changes.

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16:30 Thursday 4th July 2013

A4.19

To boldly go: Investigating co-variation in physiology and behaviour in an airbreathing fish

David J McKenzie (CNRS Montpellier, France), Thiago C Belão (UFSCar São Carlos, Brazil), Shaun S Killen (Glasgow, United Kingdom), Felipe R Blasco (UFSCar São Carlos, Brazil), Vivian M Zeraik (UFSCar São Carlos, Brazil) and F Tadeu Rantin (UFSCar São Carlos, Brazil)

Facultative airbreathing fishes may be interesting models to investigate co-variation in traits of physiology and behaviour. Although such species can maintain routine metabolic rate (RMR) by ventilating water alone, opting to breathe oxygen-rich air may allow individuals to maintain higher RMR and growth rates. This may have a direct ecologically relevant link to boldness because surfacing puts fish at much greater risk of capture by aerial and terrestrial predators. We investigated correlations among individual spontaneous air-breathing behaviour, metabolic rate, specific growth rate (SGR), and boldness, in thirty African walking catfish *Clarias gariepinus*.
The percentage of 24-hour RMR derived from airbreathing (RMR-%A) varied from 10 to >40% among individuals, being lower during daytime (RMR-%A-D, 0–35%) and higher at night (RMR-%A-N, 15–64%). The RMR-%A, RMR-%A-D and RMR-%A-N were all strongly positively correlated with 24-hour RMR itself (n=30, Spearman R = 0.626, p <0.0005 for RMR-%A). Boldness was evaluated as time required to return to airbreathing after a fearful stimulus in daylight (T-return), and was positively correlated with RMR-%A-D (n=29, Spearman R = 0.470, p <0.01). There was no correlation between standard metabolic rate (SMR) and RMR-%A or T-return. There were no correlations between SGR and any of the metabolic or behavioural variables.
Thus, individuals that air-breathed more did maintain higher RMR, and this was associated with an increased tendency to boldness. These behaviours did not, however, co-vary with individual basal oxygen demand or growth rate.

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16:45 Thursday 4th July 2013

A4.20
Ghrelin increases food intake, swimming activity and growth in juvenile brown trout (*Salmo trutta*)

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Ghrelin is a peptide that was discovered in the rat stomach 1999 as the endogenous ligand to the growth hormone secretagogue-receptor, and thus a potent stimulator of pituitary growth hormone release. Several key functions of ghrelin have been conserved during vertebrate evolution. However, ghrelin's effects on food intake are unclear in teleosts and few species have been used in functional studies on food intake and foraging-related behaviours.
Here we investigated the effects of ghrelin on activity, food intake, growth and aggressive contest behaviour in juvenile brown trout (*Salmo trutta*) using intraperitoneal implants. Food intake and swim activity were individually recorded starting from day two after implantation, and aggressive behaviour was tested on day 13 after implantation. Body mass and length were measured at the beginning of the experiment, and before and after the behavioural test. Triglycerides and lipase activity were analysed in muscle and liver; monoamine levels in telencephalon and brain stem, and NPY mRNA levels in the hypothalamus.
Ghrelin treatment was found to increase food intake, swimming activity, foraging activity and growth, without modifying lipid deposition or metabolism in liver and muscle. Furthermore, no changes were observed in monoamines and NPY mRNA levels. Ghrelin is therefore suggested to act as an orexigenic hormone regulating behaviour in brown trout.

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17:00 Thursday 4th July 2013

A4.21
Variation in animal personality and cognition

Hanne Lovlie (Linköping University, Sweden)

Over the past decade it has become apparent that animals show individual variation in behavioural responses that are consistent across time and/or context, i.e. personality. However, our understanding of the evolution of animal personality remains poorly understood, including the underlying causes, and consequences variation in personality has for the individual.
By exposing red junglefowl chicks (*Gallus gallus*) to a series of personality assays and cognitive behavioural tests, key aspects of how variation in animal personality influences cognitive processes were investigated. Proactive individuals were quicker to form routines compared to more reactive, flexible individuals. However, proactive individuals held routines longer, requiring longer time to adjust to reversal learning, and also showed weaker generalization of learned cues.
These results suggest that incorporation of individual variation in personality can improve our understanding of variation in animal learning. Additionally, combining investigation of variation in personality and cognitive processes offers the opportunity to assess how personality types interact with the environment, thus explaining observed variation in behavioural responses among individuals.

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17:15 Thursday 4th July 2013

A4.22
Linking memory formation to stress response

Sarah Dalesman (University of Exeter, United Kingdom)

The pond snail, *Lymnaea stagnalis*, demonstrates differences among but consistency within populations in their ability to form memory. Following an identical training regime some populations form memory lasting three to six hours ('standard' populations), whereas others demonstrate memory lasting five to six days ('smart' populations). This variability is consistent across generations and occurs irrespective of whether they are wild caught or laboratory reared.
Memory phenotype also correlates with the way in which *L. stagnalis* responds to environmental stress. The effects of stress on behaviour are not consistent among all stressors (i.e. 'smart' snails don't always cope better), but instead their response to stress differs dependant on the type of stress experienced. This correlation will be discussed in terms of the ability of *L. stagnalis* to demonstrate behavioural plasticity in stressful environments dependant on the phenotype they display in the absence of stress.

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Poster session – Thursday 4th July 2013

A4.23
Sexual dimorphism of locomotor performance in *Tropidurus torquatus* lizards: Relationships with body size and shape

Tiana Kohlsdorf (University of São Paulo, Brazil), Renata Brandt (University of São Paulo, Brazil), Fabio C Barros (University of São Paulo, Brazil) and Carolina Noronha (University of São Paulo, Brazil)

Sexual dimorphism (SD) is the evolutionary outcome of selection acting differently on males and females. Traditional studies on SD describe differences in size but many other morphological traits might be allometric between the sexes, implying functional consequences. Here we test the hypothesis that SD in size and shape in the lizard *Tropidurus torquatus* reflects between-sex differences in locomotor performance.
We measured morphology and performance of four locomotor activities (sprint and climbing speed, endurance and clinging force). The effects of size and shape were tested separately: we calculated SIZE extracting the first principal component of a PCA analyses performed on all morphological traits, and SHAPE was obtained correcting all linear traits by retaining the residuals of their regressions on SIZE.
As a result we found that:

- larger lizards (males) outperformed smaller ones (females) in all locomotor performances;
- lizards with larger relative foot length (males) sprinted faster; and
- those with smaller relative interlimb length (males) sprinted faster and clung harder.

Sexual selection may be playing a role in the evolution of morphology in *T. torquatus* given that better locomotor performance likely favours male lizards in typical activities of a polygenic species, such as territory defence and female acquisition, while larger trunks in females maybe associated with larger space to accommodate eggs. However, male activities in defending territories may also increase exposition to predators, suggesting a synergistic effect of sexual and natural selection in the evolution of SD in *T. torquatus.*

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Poster session – Thursday 4th July 2013

A4.24

Steroid levels of male tilapia in a mirror challenge are independent of behaviour but dependent on chemical information

João L Saraiva (Centre of Marine Sciences, Portugal), Tina Keller-Costa (Centre of Marine Sciences, Portugal), Peter C Hubbard (Centre of Marine Sciences, Portugal), Ana Rato (Centre of Marine Sciences, Portugal) and Adelino VM Canário (Centre of Marine Sciences, Portugal)

The 'challenge hypothesis' suggests that animals facing a social challenge mount an endocrine response that usually results in an increase in androgens when they succeed, and a decrease when they do not. A reported exception appears to be when Mozambique tilapia *Oreochromis mossambicus* males mount escalating fights against their own mirror image, but fail to show androgen responses. The current study aimed at establishing the basis for the apparent lack of hormonal response.
Males interacting with a mirror showed three consistent categories of behavioural response – freeze, fight or court – and no differences in circulating androgens between them after the test. In a second stage, either pulses of dominant male urine (containing a putative dominance pheromone) or control water were delivered to mirror fighting males. Males exposed to dominant male urine still displayed escalated fights, but at a significantly lower level compared to control males. However, urinary levels of 11-ketotetosterone (11KT) were higher in males that received urine pulses compared to control males. This increase is more evident when males are stimulated with dominant male urine in the absence of visual stimulus (own image). The putative urinary pheromone appears to have a releaser effect suppressing aggressive behaviour and a priming effect stimulating 11KT secretion.
Our results suggest that while winner and loser effects are highly complex, subtle sensory signals may provide the key to explain the complex link between androgens and aggressive behaviour.

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Poster session – Thursday 4th July 2013

A4.25

Great tit personality in the urban habitat: Is the personality syndrome broken down?

Juan Carlos Senar (Natural History Museum Barcelona, Spain), Sepand Riyahi (Natural History Museum Barcelona, Spain), Emilio Pagani-Nuñez (Natural History Museum Barcelona, Spain), Lluisa Arroyo (Natural History Museum Barcelona, Spain) and Mats Björklund (Uppsala University, Sweden)

Personality syndrome has been described as a suite of correlated behaviours related to exploration, aggression, predator avoidance or neophobia. It has recently been suggested that in urban habitats the correlation between variables make breakdown, but data are still sparse and conflicting. We analysed differences in personality between forest and city great tits (*Parus major*) in the area of Barcelona, by comparing their behaviour in standardized test situations and through capture/recapture.
We found urban great tits to be higher explorative and show less neophobia than forest ones, suggesting urban birds to be bolder than their forest counterparts. However, although these were general features of urban great tits, there was a lack of a correlation between these behaviours in the urban habitat. Additionally we found that urban great tits showed high levels of trap shyness and a high breath rate, which suggests that in some other respects, urban great tits are behaving as shy birds.
Analyses of *Drd4* gene showed that the differences in behaviour between urban and forest tits appeared only for heterozygote (CT) birds, but not in homozygotes (CC/TT). This suggests an advantage of heterozygotes to better adapt to the urban habitat.
Overall, results show that personality syndrome in great tits is broken down in the urban habitat, probably to allow for a better adaptation to this new habitat.

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Poster session – Thursday 4th July 2013

A4.26

Using non-invasive measures of personality, stress and water quality to determine the impact of stocking density and enrichment on fish

Lynne U Sneddon (University of Chester, United Kingdom), David CC Wolfenden (University of Chester, United Kingdom) and Katherine A Sloman (University of the West of Scotland, United Kingdom)

Relatively little is known regarding the biology of many ornamental species of fish and anecdotal evidence has suggested fish are held in unnaturally high stocking densities in barren tanks with no enrichment during import and export. This study aimed to explore whether stocking density and provision of enrichment affected boldness as a measure of personality and excretion of cortisol, a stress hormone.
We used a novel object testing paradigm which elicits no or mild stress in fish but yields data upon which fish can classified as bold or conversely fearful using the latency to approach. We also validated a non-invasive approach measuring excreted cortisol in the tank water.
Fish were held in either barren or enriched tanks under an optimum or high stocking density. Fish held in enriched conditions exhibited the same response to a novel object compared with barren fish; therefore, the provision of enrichment did not affect overall boldness. Enrichment also did not reduce cortisol excretion. Optimally stocked fish were bolder than those individuals under high stocking density. Cortisol concentrations were higher in the high stocking density treatment; therefore, high stocking density fish experience relatively poorer welfare.
This study has validated water cortisol and novel object tests as a means of measuring the status of fish thereby refining existing protocols.

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Poster session – Thursday 4th July 2013

A4.27

Synchronised breathing and fish personality

Diamanto Mamuneas (Royal Veterinary College, United Kingdom), Andrew J King (Swansea University, United Kingdom), Andrea Manica (University of Cambridge, United Kingdom) and Andrew J Spence (Royal Veterinary College, United Kingdom)

Group-living species frequently show variation between individuals. Several studies have shown that differences in 'personality' traits mediate behaviour in a social context. We consider the relationship between personality and synchrony in a species of air breathing catfish (*Corydoras paleatus*) and also demonstrate the potential for this underutilized species as a model for behavioural research.

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Poster session – Thursday 4th July 2013

A4.28

Male reproductive traits, oxidative stress and carotenoids in context of ornamentation: An experimental study in the zebra finch

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Sexual selection is the basic concept proposed to explain the evolution of elaborate male ornamentation and sexual dimorphism. However, despite more than hundred years of intensive research, basic issues remain unresolved. Among others there is one interesting question: What kind of quality do secondary ornaments honestly indicate to females? There are other interesting questions which have recently arisen about mechanisms behind the relationship between quality of individuals, ornamentation, fertility and mate choice. It is hypothesized that these characteristics are critically affected by oxidative stress which can cause individual variability. Since there is a link between carotenoids, immune system and antioxidant function we can presume a possible impact of metabolism of carotenoids in male reproductive traits such as ejaculate quality (sperm concentration, motility, percentage of normal and abnormal spermatozoa etc) in context of carotenoid ornamentation.

A wide range of various carotenoid-based ornaments exists in birds, including passerines, suggesting that birds are very useful models for these types of research. In this study we tested the effect of increased oxidative stress and dietary supplementation with carotenoids on intensity of beak colour as a main carotenoid ornament and ejaculate quality such as sperm motility in the zebra finch (*Taeniopygia guttata*).

We found a significant effect of both experimentally increased oxidative stress and dietary supplementation with carotenoids on quality of beak colouration but surprisingly we found no effect on sperm motility which is usually considered as one of the most important characteristic of ejaculate quality.

We should now ask: How much are honest signals actually honest?

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Poster session – Thursday 4th July 2013

A5 – CONSERVATION PHYSIOLOGY OF MARINE FISHES

Organized by: David McKenzie (CNRS Montpellier), Paolo Domenici (CNR Oristano) and Craig Franklin (University Of Queensland)

A5.1
Physiology, behaviour and conservation of aquatic animals

Steven J Cooke (Carleton University, Canada)

Conservation science is inherently interdisciplinary and has stimulated a number of novel interfaces including conservation genetics and conservation social science. Two of the more recent interfaces in conservation science involve physiology (i.e., conservation physiology) and animal behaviour (i.e., the behaviour–conservation interface). To date, these two interfaces have been considered separate entities, but from both pragmatic and biological perspectives, there is merit in better integrating physiology and behaviour to address applied conservation problems. Examples of potential and actual integration of physiology and behaviour to inform conservation are presented with a particular emphasis on aquatic animals.

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10:30 Friday 5th July 2013

A5.2
Long-term otolith growth biochronologies provide novel ecological and evolutionary insights into fish responses to climate change

John Morrongiello (CSIRO Marine and Atmospheric Research, Australia) and Ronald Thresher (CSIRO Marine and Atmospheric Research, Australia)

Understanding the impacts of environmental change on biological systems and making predictions of these under future environmental scenarios are key tasks facing researchers today. To do this requires good data. In a climate change context, good data can take the form of the historical record or targeted experimentation; the former relies on observing how biology has responded to past climatic variability and using this to gain insights into how it will respond to future environmental conditions, whilst the latter facilitates a mechanistic understanding of the drivers of biological change.

Unfortunately long-term biological datasets are uncommon for aquatic systems and many marine animals such as large-bodied fishes are unsuitable for experimentation. Aquatic biochronologies, generated from time-dependent information recorded in fish otoliths (ear stones) that are archived in their millions worldwide, can provide valuable long-term datasets that facilitate the development of ecological and evolutionary insights into marine and freshwater environments. This resource is, however, currently underutilized in the measurement and prediction of ecological responses to climate change, despite its potential to provide unprecedented levels of spatial and temporal detail in aquatic environments.

Using two temperate, commercially harvested, marine fishes as case studies (tiger flathead and purple wrasse), I show how within- and among-individual and population-level growth variation are related to changes in water temperature and fishery activity.

Our results indicate that projected ocean temperature rises will result in spatially differentiated changes in fishery productivity and that this is underpinned by varying levels of phenotypic plasticity and potentially micro-evolved directional change.

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11:00 Friday 5th July 2013

A5.3
Metabolic responses of free-swimming wild salmon to a simulated fisheries capture

Graham D Raby (Carleton University, Canada), Timothy D Clark (Australian Institute of Marine Science, Australia), Natalie M Sopinka (University of British Columbia, Canada), David A Patterson (Fisheries and Oceans Canada, Canada), Anthony P Farrell (University of British Columbia, Canada), Scott G Hinch (University of British Columbia, Canada) and Steven J Cooke (Carleton University, Canada)

Experimental research with physiological endpoints can be used to understand causes of mortality in fish that are captured as by-catch and released but fail to survive. In British Columbia, Canada, an endangered population of coho salmon is caught and released by multi-sector fisheries in both the marine environment and freshwater, while *en route* to spawning areas.

We used hatchery-origin coho salmon that had recently transitioned from the marine environment to examine physiological responses to simulated capture. Fish were subjected to a beach seine capture simulation in large groups at two different temperatures and for two different durations of the capture stressor. We simultaneously used a combination of surgically-implanted heart-rate biologgers, blood and tissue biopsy, and respirometry to monitor physiological recovery for 24 hpirs following capture.

Recovery of blood and white muscle metabolites was generally complete within four hours, while heart rate appeared to take somewhat longer to return to baseline. When higher temperatures were combined with longer exposure to the stressor, metabolic disturbances remained for an extended duration after release. The total energy required for coho salmon to recover from fisheries capture does not appear to be significant in the context of their overall migration energy budget.

Our data illustrate how metabolic collapse can result in by-catch mortality. Fishers should be encouraged to minimize handling stress for coho salmon by-catch, particularly at high temperatures.

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11:20 Friday 5th July 2013

A5.4

Rainbow trout telemetry in lakes reveals peak summer supra-optimal temperature habitat predicted to severely restrict heart rate and metabolic scope

Christine Verhille (University of California Davis, United States), Katja Anttila (University of Turku, Finland) and Tony P Farrell (University of British Columbia, Canada)

Biotelemetry of rainbow trout in two British Columbia lakes indicated that trout predominantly frequented warm surface waters during peak summer temperatures despite deeper, cooler and well-oxygenated water being available. In July, when lake temperatures peaked, trout almost exclusively utilized 18–20°C water. Given the temperature-dependent declines in aerobic scope at supra-optimal temperatures, we investigated the potential consequence of this behaviour by characterizing the response of maximum heart rate (fH_{max}) to acute warming. This is because temperature-dependent changes in oxygen consumption are primarily met by increased cardiac output and heart rate up to maximum values. Thus, by characterizing the thermal response of fH_{max}, identification of the Arrhenius break point temperature (ABT is potentially the optimum temperature, where aerobic scope is maximal) and the temperature where the heartbeats become arrhythmic (potentially the critical temperature) was possible. For rainbow trout, fH_{max} had an ABT of 14°C and cardiac arrhythmia began at 17°C. Therefore, for the entire month of July, trout in both lakes rarely utilized optimal temperatures and almost exclusively utilized temperatures around those predicted to trigger cardiac arrhythmia, which impairs maximum cardiac capacity for aerobic activity. Correspondingly, fish survival in these lakes decreased once the lake surfaces had warmed to 18°C and was particularly low in an unusually warm year, suggesting temperature was an important factor contributing to mortalities in these lakes. In conclusion, rainbow trout apparently selected temperatures that restricted their metabolic capacity and reduced survival in nature.

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11:40 Friday 5th July 2013

A5.5

The European silver eel (*Anguilla anguilla*) spawning migration: Marathon or sprint?

Marisa GC Vedor (Lund University, Sweden), David Righton (Cefas, United Kingdom), Julian Metcalfe (Cefas, United Kingdom) and Alan Walker (Cefas, United Kingdom)

The population and recruitment of European eels has declined dramatically over the last thirty years. Several anthropogenic threats to the eel population were identified and, in response, the EU established a management plan that imposes restrictions on exploitation. However, the impact of changes in oceanic and environmental factors may also affect the population dynamics of eels and it has been suggested that migration failure may have contributed to the collapse in eel recruitment.

We used data collected from electronic tagging experiments to map the behaviour and thermal experience of female silver eels as they migrated across the Atlantic Ocean. The eels travelled up to 50 km per day and exhibited a consistent vertical diel movement pattern, ascending to warmer shallower waters at dusk and descending into deep, colder water at dawn, from ~200–900m and 14–9°C every day. To assess energy use during the migration, we conducted respirometry experiments to determine metabolic rates at different temperatures, and extrapolated these to the recorded observations of thermal experience and swimming speed from the tagging data.

Our results provide the first estimates of energy expenditure of free-ranging eels during their oceanic migration shedding new light on the role of fat reserves during the spawning migration and the impact that trends in fat content may have on spawning success of the European eel. This application of experimental results to the eel migration success will help to establish how management measures and effective conservation plans might need to adapt in the future.

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11:55 Friday 5th July 2013

A5.6

Metabolic scope in a native and an invasive fish species: The effect of temperature

Stefano Marras (IAMC – CNR, Italy), Fabio Antognarelli (IAMC – CNR, Italy), Michel Bariche (American University of Beirut, Lebanon), Ernesto Azzurro (ISPRA, Italy), Marco Milazzo (University of Palermo, Italy), Andrea Cucco (IAMC – CNR, Italy) and Andrea Satta (IAMC – CNR, Italy), Paolo Domenici (IAMC – CNR, Italy)

The worldwide climate has warmed by 0.61°C over the past 100 years, and according to the projections made by the International Panel for Climate Change, sea surface temperatures will continue to increase globally throughout the 21st century, with dramatic consequences in the near future. Direct effects of temperature changes include distribution shifts towards higher latitudes and depths and increase in extinction rates. This is likely to be partly related to the strong effect of temperature on fish metabolism which, in turn, affects growth rate, survival, and reproduction. In the Mediterranean Sea, warming conditions are considered to be facilitating the incoming and spread of tropical invaders but so far scant information exists on the relationship between temperature and their metabolic performance. The increasing success of these species may be partly related to the fact that their optimal temperature, in terms of maximizing metabolic scope, is higher than that of indigenous species. Our objective is to determine the effect of temperature on the metabolic scope of two fish species that occupy a similar ecological niche: the native *Sarpa salpa*, and the invasive *Siganus rivulatus*, that entered the Mediterranean from the Red Sea through the Suez Canal. This latter has already colonized large areas of the basin, especially in its Eastern sectors, were it has to a large extent replaced the indigenous *S. salpa*. Our experimental results provide insight on the adaptive potential of this invader to the Mediterranean Sea and on the possible competition with its native counterpart.

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12:10 Friday 5th July 2013

A5.7

Temperature and exhaustive exercise modulate escape performance in juvenile golden grey mullet

Shaun S Killen (University of Glasgow, United Kingdom), Donald Reid (University of Glasgow, United Kingdom), Stefano Marras (National Research Council, Italy) and Paolo Domenici (National Research Council, United Kingdom)

Although several studies have investigated the consequences of temperature on fish locomotion, the majority of this work has examined effects of temperature aerobic swimming ability, while effects on anaerobic burst-type swimming have received less attention. Furthermore, the influence of temperature on the escape response following physical exhaustion – such as that which occurs while being chased by a predator – have never been investigated. High-speed pursuits by predators can cause prey exhaustion, perhaps impairing escape ability during subsequent attacks. The time required to recover escape ability is unknown and could be mediated by temperature. Juvenile mullet were acclimated to 18, 22, or 26°C (n=14 per temperature). They were then measured for standard and maximal metabolic rate (used to calculate aerobic scope), and tested for fast-start escape ability at one, two, three, four and five hours after exhaustive exercise. Aerobic scope changed only slightly across acclimation temperatures in juvenile mullet. Temperature also had little direct effect on fast-start ability, but interacts with individual aerobic scope so that at cooler temperatures, individuals with lower aerobic scopes take longer to respond after a simulated attack. After exhaustive exercise, fish generally appear limited in escape ability over the next one to two hours. This is independent of temperature, but may vary among individuals in relation to aerobic scope or standard metabolic rate. Together these results suggest that although the aerobic scope of juvenile mullet appears quite robust to changes in acclimation temperature, there may be indirect effects on the ability to escape predatory attacks.

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12:25 Friday 5th July 2013

A5.8
Metabolic shifts involved in the response to ocean acidification and warming in fish

Felix C Mark (Alfred Wegener Institute, Germany)

Hand-in-hand with ocean warming, ocean acidification is increasingly threatening life in the world's oceans. This has sparked a growing number of studies of the effects of ocean acidification and warming on water breathing, ectotherm marine animals throughout the last decade. Among those species, fish have generally been regarded as reasonably tolerant towards ocean acidification, due to their well-developed capacities for ion- and pH-regulation. As a result, there are only a handful of physiological studies on ocean acidification effects in fish, and only very few studies have dealt with metabolic regulation at the cellular level. Yet, increased costs of acid–base regulation and metabolic rearrangements may either entail adjustments of the animal's energy budget, leading to reduced growth and fecundity, or a higher total aerobic energy demand for the whole animal, reducing aerobic scope.
In this presentation, I will focus on the current knowledge of the effects of ocean acidification and warming on the cellular metabolism and molecular regulation in various ontogenetic stages of marine fish of different biogeographic origin. I shall try to indicate capacities and bottlenecks for acclimation and discuss the consequences and limitations for the whole animal level with regard to population resilience.

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13:40 Friday 5th July 2013

A5.9
Physiological, behavioural and energetic correlates of coastal marine and in-river migration of adult sockeye salmon *en route* to spawning grounds

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The reproductive migration of Pacific salmon (*Oncorhynchus* spp.) is an extremely challenging phase of their life history as they navigate from the open ocean through coastal habitats to reach natal streams. Though behaviour and physiology have been studied for the upriver migration, equivalent information for the coastal marine migratory phase has been difficult to obtain. The development of acoustic acceleration transmitters equipped with pressure sensors provided a new tool for the study of swimming activity, energy use patterns and migration depth of salmon in both marine and fresh water. Ocean migrating sockeye salmon (*O. nerka*) bound for the Fraser River, British Columbia were intercepted approximately 200 km from river entrance, tagged, and tracked as they crossed several acoustic receiver lines in coastal waters and the lower Fraser River. Data from 55 tagged fish revealed that swim speed, energy use and depth varied among migratory locales. Freshwater migration difficulty (i.e., freshwater migration distance and elevation to natal stream) was positively correlated with swim speed and energy use in the marine environment suggesting that marine swim speeds and energy use differ due to physiological and morphological traits selected for by historic migration difficulty in freshwater. Detailed assessments of individual swim speed, and depth patterns revealed a high level of inter-individual variability, though behaviours were consistent within an individual. Physiological variables (cortisol, lactate, ions, etc) were used to explain some of the inter-individual differences in behaviour and energy use. Consistent with the physiological literature, coastal migrating fish were swimming near metabolically optimal speeds.

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14:10 Friday 5th July 2013

A5.10
The intestinal barrier function of cold-adapted Antarctic fish in warmer waters

Malin Rosengren (University of Gothenburg, Sweden), Fredrik Jutfelt (University of Gothenburg, Sweden), Michael Axelsson (University of Gothenburg, Sweden), Albin Gräns (University of Gothenburg, Sweden) and Kristina S Sundell (University of Gothenburg, Sweden)

The polar regions of our planet are predicted to be severely affected by global warming. The Antarctic fish, *Pagothenia borchgrevinkihas* evolved a long time in the isolated, stabile and cold environment around the Antarctic continent. Living in a narrow temperature range, generally below 0°C, it has lost its ability to tolerate large temperature fluctuations. How will these highly cold-adapted fish cope with the projected raise in water temperature, will they be able to acclimatize and adapt? In a study conducted in Antarctica, 2011, wild caught, *Pagothenia borchgrevinki* were divided into three groups, -1.9°C (control), 4°C for 24 hours (acute) and 4°C, for three weeks (acclimated). Haematology, plasma cortisol, osmoregulatory ability and the intestinal barrier function were used as markers for stress status and condition. In the acutely exposed group it was evident that the increase in temperature acted as a stressor. In the acclimated group, habituation had occurred in the primary stress response parameters but a decrease in the intestinal barrier function was apparent. In temperate species, studies have shown that a reduction in intestinal integrity is a good indicator for chronic stress and may result in a disturbed homeostasis and increased translocation rate of pathogens. A decrease in Na^+/K^+-ATPase activity of the gills further indicates affected osmoregulatory function. *Pagothenia borchgrevinki* was clearly affected by the almost 6°C increase in body temperature, both in the acute and long term exposed groups. These changes might negatively impact the specie's ability to cope with future increases in water temperature.

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14:30 Friday 5th July 2013

A5.11
Responses of mangrove fishes to climate change

Cory D Suski (University of Illinois, United States), Aaron D Shultz (Cape Eleuthera Institute, Bahamas), Zachary Zuckerman (Cape Eleuthera Institute, Bahamas) and David P Philipp (Illinois Natural History Survey, United States)

Future climate change scenarios for tropical climates are currently predicting an increase in oceanic temperature, an increase in ocean salinity and a reduction in ocean pH. Many marine organisms appear to be quite sensitive to small changes in oceanic conditions, and, in response to changing oceanic environments, fish can experience energetically costly physiological disturbances, or they may choose to seek more favourable water conditions. Mangrove fishes reside in a dynamic and extreme environment within tropical oceans, and also play vital roles in within the marine community, but their responses to future climate scenarios has received relatively little attention. The objective of this study was to quantify the response of mangrove fishes exposed projected future oceanic conditions, and compare physiological and behavioural responses to these different environments. The water quality parameters used included an increase in salinity, a reduction in pH, elevated temperature, and a treatment that combined both increased temperature combined with reduced pH. Water conditions were manipulated by gradually adjusting ambient seawater in the direction of change that exceeds predictions by the Intergovernmental Panel on Climate Change. Blood samples were taken from each fish and analysed for indicators of stress, and behavioural responses were also quantified. Results from this experiment will contribute to our understanding of how performance, and ultimately fitness, of these fishes, will change under future climate scenarios, and which facet of future oceanic environments will be the most challenging for mangrove fishes.

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15:25 Friday 5th July 2013

A5.12

Metabolic responses in the gilthead sea bream (*Sparus aurata*): Correlations to the threshold induction temperature

Konstantinos Feidantsis (Aristotle University of Thessaloniki, Greece) and Basile Michaelidis (Aristotle University of Thessaloniki, Greece)

Seasonal increases in the sea temperature may reduce the aerobic capacity of marine fish causing reduction in their physiological performance, leading to internal hypoxia and development of oxidative stress. In the present study we investigated the seasonal metabolic and antioxidant profile in vital organs, like the heart and the liver of the Mediterranean farmed fish *Sparus aurata* during a one year acclimatization period in the field in order to identify and characterize its thermal limits. Thermal and oxidative impacts on metabolic capacities were assessed by determining the maximum activities, specific activities and kinetic properties of citrate synthase (CS) and β-hydroxyacyl CoA dehydrogenase (HOAD) as well lactate dehydrogenase (L-LDH). Oxidative stress was estimated by determining the maximum activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), xanthine oxidase (XO) and glutathione peroxidise (GPx) as well as the determination of thiobarbituric acid reactive substances (TBARS) and the activation of hypoxia induced factor Hif-1α. The obtained results, revealed specific seasonal changes in the metabolic patterns and in the oxidative stress indicators in the two examined tissues. Specifically these patterns indicated metabolic compensation and probably energy allocation during increasing ambient temperature. Moreover, elevation of Hif-1α, TBARS and succinic acid levels indicated the development of internal hypoxia and of oxidative stress.

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15:45 Friday 5th July 2013

A5.13

Physiological determinants of climate change adaptability in European seabass

Karlina Ozolina (University of Manchester, United Kingdom), Hélène Ollivier (Université de Bretagne Occidentale, France), Holly A Shiels (University of Manchester, United Kingdom) and Guy Claireaux (Université de Bretagne Occidentale, France)

The European seabass (*Dicentrarchus labrax*) is an economically important fish native to the Mediterranean and North Atlantic. Its complex life cycle involves migrations through temperature gradients influencing energetics and demands of swimming. Previous studies have shown intraspecific variation in swimming performance, hypoxia and temperature tolerance in seabass, which could include deleterious and advantageous traits under the evolutionary pressure of climate change. However, little is known of the underlying determinants of this individual variation. We investigated individual variation in temperature tolerance in 30 seabass by exposing them to a warm temperature challenge. The eight best and eight worst temperature tolerant fish were then subjected to a swimming test to determine critical swimming speed. Subsequently aerobic scope was determined for each fish using stop-flow respirometry. Finally, ventricular tissues were isolated and contractility was determined using isometric muscle preparations.

Our data show a potential trade off between temperature tolerance and swimming performance. Furthermore, temperature tolerant fish had significantly lower resting oxygen consumption, when compared to the temperature sensitive group. Although improved swimming performance is considered an adaptive trait, these findings suggest that with increasing temperatures, the best swimmers may be selected against.

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16:00 Friday 5th July 2013

A5.14

Seasonal changes of molecular and physiological indicators of the cultivated species *Pagrus pagrus*

Aglaia Ntokou (Aristotle University of Thessaloniki, Greece), Konstantinos Feidantsis (Aristotle University of Thessaloniki, Greece), Andreas Anestis (Aristotle University of Thessaloniki, Greece) and Basile Michaelidis (Aristotle University of Thessaloniki, Greece)

Red porgi, *Pagrus pagrus* is a commercial fish species that is lately cultured in Greece. In this study we investigated the seasonal changes in somatomorphometric characteristics of fish and the molecular stress responses. Individuals were sampled from an aquaculture farm, from March 2012 until January 2013. The protein content of five tissues (heart, liver, blood, white and red muscle) of fish were analysed and the data were combined with the gonadosomatic and liver somatic index. Also, fat percentage was estimated under a seasonal temperature frame. Molecular responses were addressed through the expression of heat shock proteins Hsp90, Hsp70 and the phosphorylation of stress-activated protein kinases; ERKs, p38 MAPK and JNKs and also the induction levels of ubiquitin. It appears that red porgi suffers less stress during autumn. In particular Hsp90, Hsp70 and ERKs had a significant reduction in September in red muscle, blood and heart. Nevertheless, p38 MAPK phosphorylated levels were increased and fat percentage was reduced during the same season. The higher levels of stress indicators could be a result of its reproduction period that occurs in spring and absorbs major part of its energy depends on the tissue and the temperature's limits during the hot Mediterranean summer and cold winter. We believe that the present results will provide information about better farming and conservation of the species and also some initial understanding of its favourable and stress limits to predict the physiological responses in a possible heat increase due to the climate change and ocean warming.

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16:15 Friday 5th July 2013

A5.15

DBA spectra: A method for measuring behaviour and activity-specific energy use of fish under changing conditions

Serena Wright (Cefas, United Kingdom), Julian Metcalfe (Cefas, United Kingdom) and Rory Wilson (Swansea University, United Kingdom)

The ability to predict how animals respond and make trade-offs in the face of environmental change is key to a better understanding of fish ecology. Metabolic rate is widely regarded as a 'universal currency' providing an objective measure that can be used to attribute cost to different activities. However, measuring the metabolic rate of animals in large homogenous groups or in the field can be problematic, particularly in marine fish. Dynamic body acceleration (DBA) measured using archival tags that incorporate tri-axial accelerometers has recently been shown to be an effective proxy for activity-specific metabolic rate in teleost fish, mirroring results for terrestrial animals and one species of elasmobranch. Using Atlantic cod (*Gadus morhua*) as an example, we describe a relatively simple method for deriving DBA spectra from accelerometer data. The DBA spectrum is a frequency distribution of normalized DBA over a window of time, from which statistical features can be extracted. Our results show a significant influence of temperature on the DBA spectra, with potential links to the aerobic scope of the fish. DBA spectra therefore offer the capability to understand how environmental stressors affect fish energetics, and how they then can make trade-offs to adapt with environmental change. The simple descriptive statistics from the DBA spectra also enables us to investigate the impacts of multiple stressors (temperature, hypoxia and acidification) both singly and in combination, with the method applicable to both lab-based ground-truthing studies and studies in the field.

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16:30 Friday 5th July 2013

A5.16

From physiology to phisheries: Can physiology help predict the potential impacts of environmental change fish populations, ecosystems and human society?

Julian Metcalfe (Cefas, United Kingdom), Will Le Quesne (Cefas, United Kingdom) and John Pinnegar (Cefas, United Kingdom)

Opinions on the potential impact of environmental change on marine ecosystems range from wholesale degradation through to no discernible impact and minimal consequences. Constraining this range of predictions is necessary if science is going properly to support the development of informed policy and management. The direct biological impacts of environmental change (e.g. ocean acidification, increasing temperature, decreasing oxygen) on individual organisms occur at the molecular and cellular level; however, it is the expression of these effects at population and ecosystem levels that is of concern to society. Here we consider approaches required to assess the potential impact of environmental change on fisheries, with particular emphasis on scaling up from physiological responses to populations, ecosystems and society.

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16:45 Friday 5th July 2013

A5.17

Understanding variation in behavioural response to human-induced environmental change

Andrew Sih (University of California at Davis, United States)

My talk will focus on physiology's complementary trait: behaviour. A major issue in conservation is the need to understand variation in ability of animals to cope behaviourally with human-induced rapid environmental change (HIREC: climate change, novel habitats and species, pollutants, human exploitation). Some animals are adjusting well to the modern world (some so well that they are pests), while others are falling into evolutionary traps. While there are a rapidly growing number of case studies (in marine systems and other systems) on behavioural responses to HIREC, to date, the field is largely descriptive; i.e., the field lacks explicit theory that generates predictions that empiricists can then test on variation in response to HIREC. Here, I present a theoretical/ conceptual framework and illustrate the framework using three types of models:
(1) bet hedging models to explore conditions when generalists should (or should not) respond well to HIREC;
(2) signal detection theory that yields predictions on responses to novel 'options' (predators, humans, resources); and
(3) adaptive plasticity theory that makes predictions on which organisms should shift the timing of major life history events (e.g., onset of reproduction, migration) in response to climate change.
Each model generates new predictions or insights that should apply to marine fish, as well as other organisms.

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10:30 Saturday 6th July 2013

A5.18

Ocean acidification does not compromise digestion-related metabolism in a predatory coral reef fish

Timothy D Clark (Australian Institute of Marine Science, Australia) and Jodie L Rummer (James Cook University, Australia)

The continuing rise in atmospheric CO_2 is projected to cause a decrease in ocean pH by 0.3–0.4 units within the next hundred years. Given the intricate changes in pH associated with digestion in fishes (HCl secretion from parietal cells, alkaline tide), digestion presents itself as one of the fundamental physiological processes that may be impacted most by ocean acidification.
To test this, we housed the predatory coral reef fish, blue-spotted rockcod (*Cephalopholis cyanostigma*), in respirometers at 28°C and at either control (380 µatm) or high (1800 µatm; double the current end-of-century estimate) CO_2 levels and monitored specific dynamic action (SDA) after ingestion of measured meals of sardines. While increasing meal size caused a predictable increase in each of SDA, SDA duration (range 32–80 hours), and peak oxygen consumption rate during digestion (MO_2peak) (range 2.3–5.3 mg min^{-1} kg^{-1}) in both treatment groups, we found no evidence of the high CO_2 treatment being detrimental to the SDA process. In fact, fish under high CO_2 tended to have a lower SDA for a given meal size due to a lower SDA duration (~10 hours less) without any difference in MO_2peak. These findings emphasise that coral reef fishes are adapted to significant fluctuations in CO_2 in their natural environment throughout days and seasons, although further work is required to determine whether the differences in SDA documented here translate into enhanced digestive efficiency under high CO_2.

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11:00 Saturday 6th July 2013

A5.19

The effects of aquatic acidification on growth, development and olfactory responses of early life stages of pink salmon, *Oncorhynchus gorbuscha*

Michelle Ou (University of British Columbia, Canada), Emily Lyall (University of British Columbia, Canada), Junho Eom (University of British Columbia, Canada), Sang Sun Yung (University of British Columbia, Canada), David Close (University of British Columbia, Canada) and Colin J Brauner (University of British Columbia, Canada)

In the next century, ocean acidification (OA) is predicted to have significant impacts on marine ecosystem communities and structure, yet little work has investigated the influence of CO_2 mediated acidification in freshwater (FW) systems. Pink salmon (*Oncorhynchus gorbuscha*) are the most abundant Pacific salmon and consequently of great commercial and ecological importance. In fact, they are often thought of as an indicator of ecosystem health. Unlike other salmon, pink salmon enter the ocean at a very small size (0.2 g) immediately following gravel emergence and before they are fully prepared for seawater (SW) entry. This may make them more sensitive to environmental disturbances like aquatic acidification (AA); however, this has yet to be investigated. Fish were exposed to four different pCO_2 scenarios (400, 1000, 1,600 and a diurnal oscillating treatment (400–1,600 µatm) in FW, from eyed-embryos through to yolk-sac absorption. Fish were then transferred to SW (corresponding with the time that they would naturally migrate to the ocean), and exposed to the same pCO_2 scenarios in SW for three weeks. Growth, mortality, and aerobic scope were measured weekly and olfactory responses to amino acids were measured at the end of freshwater rearing. At high pCO_2, larvae appear to have an impaired ability to sense the presence of specific amino acids in FW. The effect of the different pCO_2 scenarios on growth and aerobic scope will also be discussed.

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11:20 Saturday 6th July 2013

A5.20

Can IBMs tell us why some cod undertake vertical (feeding) migrations into hypoxic waters?

Jane W Behrens (National Institute of Aquatic Resources, Technical University of Denmark, Denmark), Stefan Neuenfeldt (National Institute of Aquatic Resources, Technical University of Denmark, Denmark) and Mikael Van Deurs (National Institute of Aquatic Resources, Technical University of Denmark, Denmark)

Deployment of data storage tags (DTSs) on Atlantic cod in the Bornholm Basin (BB) has shown that one third of the tagged population undertake vertical migrations into moderate to severely hypoxic water, presumably to feed. In-depth analysis of these movement patterns however reveals no distinct temporal pattern over longer periods, neither among nor within individuals. Hence, we hypothesized that such behavioural decision is minute-by-minute, driven by *ad hoc* prey availability and the internal physiological state of the fish. We here explore the potential of IBMs (Individual Based Models) as mean to understand how temporally highly resolved physiological processes may explain how state-dependent trade-offs drive foraging behaviour in these fish. By combining information on: (i) the unique oxygen conditions prevailing in the BB, (ii) DST-derived temperature and depth experienced by individual cod in the BB and (iii) cod physiology and behaviour ranging from swimming performance and thermal responses to detailed models of gut evacuation rate, specific dynamic action and the cost of repaying a post hypoxic oxygen debt, we aim at reproducing in real time, digestion rate, energy assimilation and exploitation of metabolic scope for a cod. Unknowns are the frequency of prey capture rate and prey types, hence, these variables will be subject to a range of different IBM scenario run. To ensure realism and relevance of the scenarios we will use data from 12,000 stomachs collected individually to estimates meal frequency, size of an average meal, prey composition and related coefficients of variation as input to the IBMs

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11:40 Saturday 6th July 2013

A5.21

Impacts of hypoxia on three commercial (Atlantic cod, Greenland halibut, northern shrimp) and one threatened (spotted wolffish) cold-water marine species

Denis Chabot (Maurice-Lamontagne Institute, Canada)

Hypoxia (low dissolved oxygen, DO) is a permanent characteristic of the deep water (>150 m) in the Estuary and Gulf of St. Lawrence, eastern Canada. Conservation physiology techniques have shown that one commercial species, Atlantic cod (*Gadus morhua*), and a threatened species, the spotted wolffish (*Anarhichas minor*), are sufficiently sensitive to hypoxia that some parts of this ecosystem are no longer adequate habitat for them (ambient DO <30% sat.). Adult Greenland halibut (*Reinhardtius hippoglossoides*) and male northern shrimp (*Pandalus borealis*), two important commercial species, are more tolerant (critical levels ~10% sat.). However, juvenile halibut and female shrimp have tolerance levels of ~15% sat., close to ambient levels in the Estuary (18–25% sat.). All four species experience ambient DO levels that limit their aerobic scope (the difference between maximum and minimum metabolic rates), but only cod and wolffish have been shown to eat less and grow more slowly at these DO levels in the laboratory. Cod show some avoidance of hypoxic water in the area, but a large proportion of the stock is found at DO <70% sat., and productivity of the stock could be affected. Reduced productivity and possibly recruitment may impair the recovery plan for the spotted wolffish. A further drop in DO of only a few % sat., a possible outcome of climate change, could have strong repercussions on Greenland halibut and northern shrimp in the Estuary and Gulf of St. Lawrence.

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11:55 Saturday 6th July 2013

A5.22

Exposure to environmental hypoxia during larval stage affects assimilation and growth in juveniles of the seabass (*Dicentrarchus labrax*)

José-Luis Zambonino-Infante (Ifremer, France), Alexia Dubuc (Ifremer, France), Pierre Queau (University of Brest, France), Gwenaelle Vanderplancke (Ifremer, France), David Mazurias (Ifremer, France), Nicolas Le Bayon (Ifremer, France), Patrick Quazuguel (Ifremer, France) and Guy Claireaux (University of Brest, France)

Predicted changes in world oceans are likely to be associated with increased severity and occurrence of hypoxic episode, particularly in marine coastal areas. These shallow ecosystems also provide nursery areas where larvae from a large number of species settle from a planktonic life to shelter and forage for food. The objective of the present study was to investigate whether oxygenation conditions at larval stage, via irreversible plastic response during ontogeny, can affect traits of physiological performance at later, juvenile stage. European seabass larvae (*Dicentrarchus labrax*; 30 days post-hatch) were attributed to two experimental groups and either maintained under normoxic conditions (24°C) or exposed to a hypoxic episode (10 days at 24°C and 40% air saturation). During the following seven months larvae were kept under similar conditions (normoxia, 20°C). Experimental results showed that juveniles that had been exposed to a hypoxic episode at larval stage displayed lower growth (-8%) than fish that had been maintained under normoxia. Although no difference in standard metabolic rate, active metabolic rate and maximal swimming speed was observed between the two groups, postprandial excess oxygen consumption was 8% lower in the hypoxia-exposed fish. Calorimetric measurements showed this potentially impaired assimilation was associated with faeces energy contents that were 8–9% higher in the hypoxia-exposed than hypoxia-naive fish.

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12:10 Saturday 6th July 2013

A5.23

Intraspecific variation in hypoxia tolerance and physiology of two marine, moronid fishes: Implications for an increasingly hypoxic world

Jay A Nelson (Towson University, United States), Guy Claireaux (Institut Universitaire Européen de la Mer, France), Felix C Mark (Alfred Wegener Institute, Germany) and Genine K Lipkey (Towson University, United States)

Studying intraspecific variation of marine fish physiology and its relation to hypoxia tolerance could provide clues as to how fish will deal with expanded hypoxic zones. We investigated individual variation and repeatability of hypoxia tolerance and its relationship to physiology and behaviour in two moronid fishes threatened by coastal hypoxia expansion, European seabass (ESB) and striped bass (SB). We measured maximum anaerobic speed at exhaustion (U_{CAT}), gait transition speed between aerobic and anaerobic swimming, swimming efficiency, routine metabolic rate, post-U_{CAT} metabolic rate and aerobic scope in ESB. In SB, we examined how swimming and position in dominance hierarchies influenced hypoxia tolerance. After their physiology experiments, ESB were also released into artificial estuaries for five months at densities that ensured competition for natural forage but were not exposed to predation. Hypoxia tolerance in static tanks was significantly repeatable at various scales in both species, but demonstrated significant phenotypic plasticity in SB only. Swimming predictably lowered hypoxia tolerance, but interestingly, there was no correlation between individual swimming versus static hypoxia tolerance. Position in a dominance hierarchy was significantly repeatable but unrelated to hypoxia tolerance. Metabolic rate after the U_{CAT} test varied substantially among individuals, was inversely related to hypoxia tolerance, indicative of swimming pattern during the U_{CAT} test and was predictive of an animal being one of the 40% that survived the estuaries.

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12:25 Saturday 6th July 2013

A5.24

Physiology, behaviour, life history traits and fitness – how do they influence each other?

Christian Jørgensen (Uni Research, Norway)

Physiology is not only a constraint for the performance of organisms but also a flexible set of mechanisms by which the organism can respond to changes in its environment. Physiology is therefore likely to co-evolve with behavioural strategies and life history traits, but how do these interact and influence each other in determining fitness? In this talk I will use two theoretical fish models to illustrate some key points. The first model studies fish behaviour in water columns with hypoxia; it is based on bioenergetics and optimizes behavioural strategies for choice of depth and activity level. In order to thrive, these fish need to consider their energetic budget that allows growth and reproduction, as well as their oxygen budget. Here a rather detailed formulation of respiration physiology is crucial for understanding behaviour, and it turns out hypoxia is not always bad but can provide important refugia under certain circumstances. The second model describes adaptations of fish populations to climate change. In contrast to the common view that physiology defines performance and strict temperature windows, we use fitness considerations to optimize foraging behaviour and life history traits. This model makes it easier to see how physiological adaptations are part of a suite of traits that have co-evolved through natural selection, and predicts that fish populations may be more flexible and have a larger repertoire of possible adaptations to keep pace with climate change.

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13:45 Saturday 6th July 2013

A5.25

Will ocean acidification affect the physiology of predatory reef fish?

Jodie L Rummer (James Cook University, Australia), Sjannie Lefevre (University of Oslo, Norway), Göran E Nilsson (University of Oslo, Norway), Sue-Ann Watson (James Cook University, Australia), Jonathan M Wilson (CIMAR Porto, Portugal), Philip L Munday (James Cook University, Australia) and Timothy D Clark (Australian Institute of Marine Sciences, Australia)

Ocean pH is projected to decrease 0.3–0.4 units by 2,100 with increasing atmospheric CO_2, yet the consequences of declining pH and rising pCO_2 for fishes remain poorly understood. We exposed blue-spotted rockcod, a medium-sized predatory coral reef fish, to 400 µatm (control), 900 µatm (projected end-of-century), or 1,800 µatm pCO_2. We examined oxygen consumption rates at rest $MO_{2\ Rest}$) and following maximal exercise MO_{2Max}) as well as blood and tissue parameters indicative of metabolic and/or pH/ion balance stress. Immediately upon CO_2 exposure, fish decreased $MO_{2\ Rest}$, but after 48 hours, $MO_{2\ Rest}$ was ~40% lower than control, regardless of treatment. After seven days exposure, $MO_{2\ Rest}$ had increased but was still 14% (900 µatm-exposed) and 28% (1,800 µatm-exposed) lower than control fish. Significant patterns were not detected in MO_{2Max} or aerobic scope. Rockcod are ambush predators living within the reef matrix; peak activity and feeding occur just after dawn and before dusk, whereas activity is minimal several hours before sunrise. CO_2 levels fluctuate diurnally on coral reefs, being lowest by day and highest at night, peaking just before dawn due to accumulated CO_2 from reef respiration. Diurnal CO_2 fluctuations may play a role in signalling metabolism, and here we begin to unravel physiological mechanisms involved. As our data suggest, end-of-century CO_2 levels may not overtly impact the metabolic physiology of this species, possibly reflecting adaptation to natural CO_2 fluctuations of their microhabitat and their activity patterns. Interactions between ocean acidification and predator-prey dynamics remain largely unexplored but will be pivotal to the continued health of coral reef ecosystems.

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14:15 Saturday 6th July 2013

A5.26

Shrinking of fishes exacerbates impacts of global ocean changes on marine fishes

William WL Cheung (University of British Columbia [UBC], Canada), Jorge L Sarmiento (Princeton University, United States), John Dunne (National Oceanic and Atmospheric Administration, United States), Vicky Lam (UBC, Canada), ML Deng Palomares (UBC, Canada), Reg Watson (University of Tasmania, Australia) and Daniel Pauly (UBC, Canada)

Changes in temperature, oxygen content and other ocean biogeochemical properties directly affect the eco-physiology of marine water-breathing organisms. Previous studies suggest that the most prominent biological responses are changes in distribution, phenology and productivity. Both theory and empirical observations also support the hypothesis that warming and reduced oxygen will reduce body size of marine fishes. However, the extent to which such changes would exacerbate the impacts of climate and ocean changes on global marine ecosystems remains unexplored. Here, we employ a model to examine the integrated biological responses of over 600 species of marine fishes due to changes in distribution, abundance and body size. The model has explicit representation of eco-physiology, dispersal, distribution, and population dynamics. We show that assemblage-averaged maximum body weight is expected to shrink by 14–24% globally from 2,000 to 2,050 under a high emission scenario. About half of this shrinkage is due to change in distribution and abundance, the remainder to changes in physiology. The tropical and intermediate latitudinal areas will be heavily impacted, with an average reduction of more than 20%. Our results provide a new dimension to understanding the integrated impacts of climate change on marine ecosystems, from ecophysiology to ecology.

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14:35 Saturday 6th July 2013

A5.27

A physiology-based, ecologically relevant assessment of fish health following an oil spill and its treatment with dispersant

Guy Claireaux (University of Brest, France), Natascha Ouillon (University of Brest, France), Florian Mauduit (University of Brest, France), Nicolas Le Bayon (Ifremer, France), Stéphane Le Floch (CEDRE, France), Paolo Domenici (CNR, Italy) and Anthony P Farrell (University of British Columbia, Canada)

The worldwide remedy to treat oil spills in coastal ecosystems with chemical dispersants is controversial due to the absence of an adequate methodology to provide reliable predictions of how dispersant-treated oil affects population-level performance that has ecological relevance. The primary objective of the present study was to examine and compare the effects of exposure to untreated oil, chemically dispersed oil or dispersant alone, upon the compensatory ability of fish to environmental challenges. Performance was measured in experimental population of 1,200 PIT-tagged, juvenile European seabass using three high-throughput, non-lethal challenge tests that measured an individual's maximum swimming performance, hypoxia tolerance and thermal sensitivity as surrogates for their capabilities to face natural contingencies. Once characterized, fish were then challenged with exposure to oil, dispersant, or to mixture of both for 48 hours, or left as untreated controls. Individual growth was monitored during the following 6 weeks, after which the three challenge tests were repeated. Our results revealed that the response of control fish to the challenge tests was variable among individuals but temporally stable (repeatable). While exposure to the chemical dispersant alone did not negatively affect fish growth or performance, exposure to oil alone did. Growth, swimming speed, incipient lethal oxygen saturation and incipient upper lethal temperature all changed significantly, and these impairments were exacerbated in the group exposed to a mixture of oil and dispersant. These data suggest that although dispersant did not affect fish performance, it may aggravate the detrimental effects of petroleum hydrocarbons on seabass.

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14:55 Saturday 6th July 2013

A5.28

Physiological-based projections of habitats suitable for temperate marine fish larvae

Myron A Peck (University of Hamburg, Germany), Klaus Huebert (University of Hamburg, Germany), Marta Moyano (University of Hamburg, Germany) and Marc Hufnagl (University of Hamburg, Germany)

Adequate conservation and management of marine fish requires knowledge on the processes affecting the productivity of different populations, particularly the habitat characteristics essential for survival of early life stages. We discuss our attempts to gain a physiological-based cause-and-effect understanding of factors affecting the early life stage dynamics of North Sea fishes through combining field survey data, laboratory experiments and biophysical modelling simulations. We provide examples of physiological-based model projections of 'winners' and 'losers' in light of climate-driven changes in larval fish nursery grounds. These estimates include:
(1) the strength of connectivity to near-shore nursery areas (larvae of plaice);
(2) the location of habitats supporting population shifts to higher latitudes (larvae of European anchovy); and
(3) climate-driven changes in overwintering survival and recruitment success (larvae of Atlantic herring).
Key, physiological-based data needed to make more robust projections are discussed from measurements of biochemical condition (e.g. nucleic acids) in field-caught individuals, to metabolic and behavioural responses of individuals to key environmental factors.

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15:35 Saturday 6th July 2013

A5.29

The role of lagoons in metapopulation function of the gilthead seabream (*Sparus aurata*) in the Gulf of Lions

Jennifer Tournois (Ecologie des systèmes marins côtiers – UMR5119, France), Franck Ferraton (Ecologie des systèmes marins côtiers – UMR5119, France), Elody Isnard (Ecologie des systèmes marins côtiers – UMR5119, France), Audrey M Darnaude (Ecologie des systèmes marins côtiers – UMR5119, France), Catherine Aliaume (Ecologie des systèmes marins côtiers – UMR5119, France) and David J McKenzie (Ecologie des systèmes marins côtiers – UMR5119, France)

Large coastal lagoons in the Gulf of Lions (NW Mediterranean) are occupied as summer feeding grounds by highly prized fish species, such as gilthead seabream *Sparus aurata*. These species breed offshore in winter; lagoons are colonized by large numbers of post-larvae in spring, indicating that they are important nurseries for juveniles. This study aimed to understand the role of the four largest lagoons in local metapopulation function of *S. aurata*. We investigated the hypothesis that the lagoons that permit the best growth rates, and produce juveniles in best physiological condition prior to their first winter offshore, will make the greatest contribution to adult stocks in the local fishery. We sampled juveniles from each lagoon at the end of their first summer and, using otolithometry, found significantly higher daily growth rates in juveniles inhabiting two warm, shallow, brackish lagoons compared to two colder, deeper more saline lagoons. These same juveniles had higher condition factor and muscle lipid stores. Using multi-elemental otolith microchemistry signatures, and random forest statistics, we can assign juveniles to their lagoon of origin with ~80% accuracy based on three years' data. We have sampled 120 adults offshore and have measured the microchemical composition of the portion of their otolith formed during their first summer. We are identifying lagoon signatures to investigate the hypothesis that the shallow warm brackish lagoons are heavily represented. This research will contribute to the conservation of stocks of this highly prized species, and of key habitats for the completion of its life cycle.

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15:50 Saturday 6th July 2013

A5.30

Riding the wave: Swimming performance and phenotypic plasticity in a coral reef fish

Sandra A Binning (Australian National University, Australia), Dominique G Roche (Australian National University, Australia), Albert FH Ros (Université de Neuchâtel, Switzerland) and David Nusbaumer (Université de Neuchâtel, Switzerland)

Waves are responsible for structuring the distribution of species across shallow marine habitats, but how do widespread species cope with a range of flow environments? Here, we show that previously documented differences in the swimming performance of a coral reef damselfish living across different flow environments are a plastic response to water flow.
Using a split-brood rearing experiment with wild-caught *Acanthochromis polyacanthus* broods, we found that critical swimming speed, maximum metabolic rate, and aerobic scope are higher in fish reared in experimental wave treatments compared to fish reared in experimental calm treatments regardless of parental phenotype. Blood sampling revealed the likely mechanism responsible for such differences: fish from wavy treatments had a higher oxygen carrying capacity (blood haematocrit) than those reared in the calm treatments. Fin shape was not different among any treatments suggesting that training alone is not responsible for the morphological differences observed across natural populations of this species.
Our results suggest that exposure to water motion is sufficient to induce plastic physiological changes in this species, which may be a primary explanation for its widespread distribution across the Great Barrier Reef and Coral Sea.

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16:05 Saturday 6th July 2013

A5.31

Eels with balls – the effect of additional drag on anguilliform swimming physiology, kinematics and behaviour

Christian Tudorache (Leiden University, Netherlands), Eric Burgerhout (Leiden University, Netherlands) and Guido Van den Thillart (ZF Screens, Netherlands)

The migration and reproduction of the European eel is an unsolved mystery. The smallest larvae were found in the Sargasso Sea, but never a mature eel. Tracking eels on their spawning migration would provide a wealth of new information. Many attempts to tag eels on their spawning migration were unsuccessful in the past, because of interfered of tracking devices with natural swimming behaviour. Available devices have a positive buoyancy and high drag. Additionally, eels are economically extremely efficient swimmers, due to minimal drag. Thus far no studies were performed to analyse the interference of external drag with the anguilliform swimming mode.
In the present study, we investigated the swimming capacity, behaviour and kinematics of migrating silver eels, with emphasis on the effect of drag force of external tracking devices on swimming mode and costs. The results show a significant effect of even low drag forces and the place of attachment on multiple energetic and biomechanic aspects of anguilliform swimming and the swimming behaviour was altered.
The present study gives a first analysis of the effect of external tagging on migrating eels and provides threshold values for the application in the field.

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16:20 Saturday 6th July 2013

A5.32

Influence of n-3 highly unsaturated fatty acids on swimming performance and energetic metabolism of the golden grey mullet (*Liza aurata*)

Marie Vagner (Université la Rochelle [UlR] – CNRS, France), José-Luis Zambonino-Infante (Ifremer PFOM, France), Emmanuel Dubillot (UlR – CNRS, France), Hervé Le Delliou (Ifremer PFOM, France), Natascha Ouillon (UlR – CNRS, France), David Mazurais (Ifremer PFOM, France), Nathalie Imbert (UlR – CNRS, France), David Akbar (UlR – CNRS, France), Marie Durollet (UlR – CNRS, France) and Christel Lefrançois (UlR – CNRS, France)

N-3 highly unsaturated fatty acids (n-3 HUFA) are weakly synthesized *de novo* and must consequently be supplied by food. In the natural environment, the main source of n-3 HUFA is provided by microalgae. Availability is subject to change as part of variability in environmental conditions and global change. As n-3 HUFAs (particularly EPA and DHA) are the main components of cell membranes in fish, their availability in diet can lead to variability in membrane composition, and consequently in physiological performance. The aim of this study was to evaluate the effect of n-3 HUFA dietary content on maximal swimming speed U_{crit} and associated oxygen consumption of golden grey mullet (*Liza aurata*), a microalgae grazer present in coastal areas. Two replicated groups of 20°C-acclimated fish were fed on a rich (1.0% EPA + DHA on dry matter basis, DMB) or a poor-n-3 HUFA (0.1% EPA+DHA on DMB) diet for four months, and were called HH and LH groups respectively. LH groups showed a 30% lower active metabolic rate and aerobic capacity than HH groups, as well as a lower growth rate (n=11 per condition). However, U_{crit} was similar in both groups. These results suggest low levels of n-3 HUFA in diet have an influence on some aerobic physiological functions of *Liza aurata*. The results will be discussed, in the context of evaluating the adaptive capacity of this species in a variable environment.

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16:35 Saturday 6th July 2013

A5.33

Effects of salinity challenge on ion regulation in estuarine migrating sea lamprey, *Petromyzon marinus*

Diogo Ferreira-Martins (CIIMAR-CIMAR, University of Porto [UoP], Portugal), Justyna Kopecka-Pilarczyk (CIIMAR-CIMAR, UoP, Portugal), Stephen D McCormick (Conte Anadromous Fish Research Center, United States), João Coimbra (CIIMAR-CIMAR, UoP, Portugal) and Jonathan M Wilson (CIIMAR-CIMAR UoP, Portugal)

The sea lamprey, *Petromyzon marinus*, is an anadromous species in which adults re-enter freshwater and migrate upstream for terminal spawning. The aim of this study was to determine the capacity for marine osmoregulation in late, upstream migrants by characterizing the morphological and physiological effects of salinity challenge from a molecular perspective. Salinity was gradually raised until a final salinity of 17.5‰. A number of relevant blood and intestinal parameters were measured to assess ionoregulatory and biochemical changes and the expression of key ion-transport related proteins by immunoblotting [Na^+/K^+-ATPase (NKA), vacuolar-type H^+-ATPase, carbonic anhydrase, and Na^+:K^+ :$2Cl^-$ contransporter]. NKA activity was measured in addition to oxidative stress indicators. The upper lethal salinity limit was determined to be >17.5‰ in the freshwater migrating adult lamprey with clear osmoregulatory failure, fish being unable to regulate Na^+ and Cl^- levels with plasma and intestinal fluid approaching environmental concentrations (osmoconforming and failure of drinking mechanism, respectively). This was accompanied by a significant drop in haematocrit (37% to 1%) and plasma lactate concentrations indicating haemolytic anaemia. Higher plasma [ALT] indicated tissue damage that correlates with oxidative damage to liver (high lipid peroxidation and GST activity, and lower [GSH]). No ion transport protein response to salinity was detected in gill, intestine or kidney by immunoblotting. The only potentially adaptive ionoregulatory response was an increase in Na+ /K+-ATPase activity in mid-intestine. FCT grant PTDC/MAR/98035/2008

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16:50 Saturday 6th July 2013

A5.34

Finding the best metabolic rate estimates in a coral reef fish

Dominique G Roche (Australian National University, Australia), Sandra A Binning (Australian National University, Australia), Yoland Bosiger (James Cook University, Australia), Jacob L Johansen (James Cook University, Australia) and Jodie L Rummer (James Cook University, Australia)

Metabolic rates of aquatic organisms are estimated from measurements of oxygen consumption rates (MO_2) through swimming and resting respirometry. Few studies have tested whether they yield comparable results. We examined whether 2 fundamental MO_2 measures, standard metabolic rate (SMR) and maximum metabolic rate (MMR), vary based on the method employed. Ten bridled monocle bream (*Scolopsis bilineatus*) were exercised using: (1) a critical swimming speed (U_{crit}) protocol; (2) a 15-minute exhaustive chase protocol; and (3) a 3-minute exhaustive chase protocol followed by brief (one minute) air exposure. Protocol (1) was performed in a swimming respirometer whereas protocols (2) and (3) were followed by resting respirometry. SMR estimates in swimming respirometry were similar to those in resting respirometry when a 3-parameter exponential or power function was used to extrapolate the swimming speed–MO_2 relationship to zero swimming speed. MMR using the U_{crit} protocol was 36% higher than MMR derived from the 15-minute chase protocol and 23% higher than MMR using the 3-minute chase 1-minute air exposure protocol. For strong steady (endurance) swimmers, such as *S. bilineatus*, swimming respirometry can produce more accurate MMR estimates than exhaustive chase protocols as oxygen consumption is measured during exertion. When swimming respirometry is impractical, exhaustive chase protocols should be supplemented with brief air exposure to improve measurement accuracy. Caution is warranted when comparing MMR estimates obtained with different respirometry methods unless they are cross-validated on a species-specific basis.

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Poster session – Friday 5th July 2013

A5.35

In vitro study of urea and ion transport in gastrointestinal tract of dogfish shark (*Squalus acanthias*): The influence of feeding

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In vitro gut sac preparations made from cardiac stomach (stomach-1), pyloric stomach (stomach-2), intestine (spiral valve), and colon were used to examine the impact of feeding on GI tract transport processes in dogfish. Preparations were made from fasting (1–2 weeks) or at 24–48 hours after voluntary feeding (3% ration) animals. Sacs were incubated under initially symmetrical conditions with dogfish saline on both surfaces. Results confirmed feeding caused increases in H^+ secretion in both stomach sections, but an increase in Cl^- secretion only in stomach-2. Na^+ absorption occurred in both stomach sections after feeding. All sections of the tract absorbed water; the intestine strongly absorbed Na^+ and Cl^- regardless of feeding condition. Feeding increased water absorption in the intestine. However, K^+ was secreted in the intestine in both fasted and fed preparations. Increased intestinal water absorption occurred despite net osmolyte secretion into the mucosal saline. The largest changes occurred in urea and CO_2/HCO_3^- fluxes. In fasted preparations, urea was absorbed at a low rate in all sections except the intestine, where it was secreted. Feeding caused a marked switch to net urea absorption. This intestinal urea transport occurred actively, establishing a large serosal-to-mucosal concentration gradient; as comparable to reabsorption rates reported at gills and kidney. Feeding greatly increased intestinal CO_2/HCO_3^- secretion; above rates in marine teleosts (if interpreted as HCO_3^- transport). Phloretin (applied mucosally) completely blocked intestinal urea absorption and CO_2/HCO_3^- secretion caused by feeding, but had no effect on Na^+, Cl^-, or water absorption. NSERC; IWS-BOF Grant

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Poster session – Friday 5th July 2013

A5.36
How can physiology improve species distribution models in marine organisms?

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Species distribution models (SDMs) are central in exploring changes in biodiversity. The reliability of predictions of SDMs has been questioned because models often lack a physiological underpinning and rely on assumptions that may be unrealistic under climate change. In this study we explore how information on the limits of thermal tolerance can improve our ability to predict the effects of climate change and get us closer to the fundamental niche of the species.
Using cephalopods as a model group, we determined in the lab the window of critical temperatures for different life stages (embryos, hatchlings and adults) and study how this information can improve our predictions for range shifts under a climate change scenario. The resulting framework may pave the way towards a better modelling of marine species distributions under climate change.

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Poster session – Friday 5th July 2013

A5.37
Effect of estrogen pollution on plasma melatonin and thyroxine in round goby (*Neogobius melanostomus*) in different periods of reproductive cycle

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The proper functioning of fish endocrine system, crucial for normal development, growth and reproduction, may be disrupted by compounds, which enter aquatic ecosystems with sewage of various origins. They belong to EDCs (endocrine disrupting chemicals) – exogenous compounds, which mimicking action of endogenous hormones can stimulate, block or modify their synthesis and metabolism in organism.
One of the most common pollutants is 17β-estradiol (E2), an estrogenic endocrine disruptor, which may pose a serious threat to marine organisms, especially these inhabiting inshore waters. The aim of this study was to investigate an influence of intermittent exposure to exogenous E2 on endocrine system of mature males and females of round goby (*Neogobius melanostomus*) in different periods of reproductive cycle. For this purpose, round gobies were bath-exposed to 200 µg E2 /l for two hours on three consecutive days at five-day intervals. The studies were conducted in pre- and post-spawning season.
At the end of each 27-day experiment, plasma samples were collected from all fishes. Changes in plasma levels of melatonin (Mel), an important antioxidant and element of fish defence system, and thyroxine (T_4), hormone of thyroid gland crucial for growth, development and reproduction, were investigated. Plasma hormone concentrations were measured by radioimmunoassay (RIA).
The changes of plasma Mel and T_4 levels in fishes exposed to exogenous E2 were different in pre- and post-spawning season. These results provide new information on response of fish endocrine system to important estrogenic pollution present in aquatic ecosystems.

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Poster session – Friday 5th July 2013

A5.38
Identification of seasonal migration, vertical activity and thermal experience of Greenland halibut *Reinhardtius hippoglossoides* (Walbaum) in west Greenland waters

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With its plentiful numbers along the Greenland west coast Greenland halibut have rendered a socio-economically important resource for the Greenlandic people for more than one century. This traditional fishery is centred in a small area off Ilulissat (Disko Bay) where up to 8–9,000 t are caught annually, making it one of the most condensed fisheries in the world. Recent studies of halibut along the continental slope between Norway and Spitsbergen has documented extensive vertical activity and use of the pelagic environment. Current stock assessment on halibut in West Greenland waters is based on data from bottom gears. Consequently, abundance estimates may be biased if the pelagic zone is frequently used. Identification of local seasonal migrations is likewise important in order to obtain unbiased biomass estimates. Temperatures along the west Greenland coast changed dramatically in the 1990s and a warm water pulse arrived in Disko Bay in 1997. This sub-surface pulse suggestively flooded (and may still remain in) the Ilulissat Icefjord, a deep-ocean fjord covered by one of the world's largest outlet glaciers. Using data storage tags we describe seasonal migrations of halibut into the permanently ice-covered Icefjord and how this relates to thermal conditions, as compared to when residing in the Disko Bay. Results are compared to a unique temperature profile from the Icefjord, and to annual temperature profiles obtained in the Disko Bay; we thus show how fish can be used as 'measuring tool' in harsh environments. Finally, we describe and discuss vertical activity patterns in the two localities

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Poster session – Friday 5th July 2013

A5.39
Global warming: Will jumping snails prevail?

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Ocean temperature has been predicted to increase at an unprecedented speed during this century. Regarding the negative impacts of global warming, tropical ecosystems, such as coral reefs, deserve particular attention because their inhabitants are already close to their maximum tolerable temperatures. The humpbacked conch *Gibberulus gibberulus* is found in the intertidal zones of islands of the Great Barrier Reef, and may often be exposed to high temperatures. During these periods the snails are probably at the edge of their performance, and may be less able to withstand extremes, when these are exaggerated by global warming. Here we investigated the effect of increased temperature on resting and active oxygen consumption rates (MO_2) in these snails, both after acclimation (>1 week at 27 and 33°C) and during acute exposure to elevated temperature (34°C and 37°C). When predator odour was used to induce jumping in the respirometer, *G. gibberulus* was able to increase its MO_2 three to four times, an impressive scope for a snail, being similar to that of fish. Resting MO_2 increased with temperature, and the change was only partly counteracted by acclimation. The snails jumped more and faster at high temperature, causing an increase in maximum MO_2. This resulted in maintained absolute scope and reduced factorial scope. These results indicate that the humpbacked conch may be relatively insensitive to global warming, at least when compared to their piscine neighbours, although further research is needed to evaluate how rising temperatures are tolerated when coinciding with the predicted increase in CO_2.

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Poster session – Friday 5th July 2013

A5.40

Elevated CO_2 reduces copper-induced genotoxicity in shore crabs (*Carcinus maenas*)

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Marine animals acutely subjected to seawater with elevated CO_2 partial pressure experience a corresponding extracellular acidosis. Many fish and crustaceans are able to regulate these acid–base perturbations by the elevation of extracellular bicarbonate ions (HCO_3^-). Many animals can use this mechanism to completely restore normal blood pH well within 24 hours of exposure to elevated CO_2. We provide evidence that the acid–base regulatory responses to high CO_2 may provide a hitherto undocumented protective effect against the genotoxic impact of copper.

Shore crabs (*Carcinus maenas*) were exposed to four experimental treatments over 14 days: (1) Control (CO_2 390 ppmv), (2) high CO_2 (CO_2 1200 ppmv), (3) Copper (CO_2 390 ppmv + copper 6.3 ug/l), and (4) combined CO_2 and Copper (1,200 ppmv + 6.3 ug/l, respectively). Analysis of haemolymph acid–base variables (pH, TCO_2) on day 14 revealed complete pH regulation due to a ~4 mM elevation in HCO_3^- ions. Assessment of haemocyte DNA damage using Comet assay showed increased DNA damage in the copper treatment compared with control animals. Most notably, there was a significant reduction in single strand breaks in the crabs exposed to copper and high CO_2 simultaneously compared with the copper only treatment.

We suggest that elevated haemolymph [HCO_3^-], resulting from the acid–base regulatory response to high CO_2, has the capacity to complex with Cu^{2+} forming less bioavailable species and thereby reduce copper toxicity. This work will be repeated using seabass (*Dicentrachus labrax*) to establish whether this protective effect is found in taxonomically dissimilar acid–base regulating species.

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Poster session – Friday 5th July 2013

A5.41

The effect of hypoxia on gut carbonate production in the European flounder (*Platichthys flesus*)

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The intestinal precipitation and excretion of solid calcium carbonate crystals by marine teleosts forms a key part of their osmoregulatory strategy. When put into a global context, the surprisingly high rate of calcium carbonate production by fish has potentially significant implications for our understanding of ocean chemistry and carbon cycling as well as carbonate sediment budgets and geological records. Recently it has been suggested that fish may contribute up to 45% of global new oceanic calcium carbonate. Understanding how environmental factors such as pO_2 affect carbonate production by fish is important in order to accurately model the role of fish in the marine inorganic carbon cycle of the past, present and future. According to our hypothesis, calcium carbonate production should increase as pO_2 declines due to increased ventilation volume causing an enhanced water loss across the gills from the blood to the hyperosmotic seawater. This is predicted to result in a compensatory increase in drinking rate which in turn would supply the gut with more seawater Ca^{2+} to be precipitated as carbonates. However, in experiments on European flounder (*Platichthys flesus*), we found no evidence of increased calcium carbonate excretion rates under hypoxia down to 5.5 kPa despite metabolic rate being maintained by a 1.6-fold increase in ventilatory frequency as predicted. Possible explanations include flounder reducing their osmotic permeability as an acclimatory response to hypoxia. Alternatively, a reduction in blood pCO_2 during hypoxia (due to hyperventilation) may limit supply of HCO_3^- to the gut for precipitation of ingested Ca^{2+} ions.

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Poster session – Friday 5th July 2013

A5.42

Metabolic plasticity in response to changing abiotic factors in Antarctic fish and cephalopods

Anneli Strobel (Alfred Wegener Institute, Germany), Hans O Pörtner (Alfred Wegener Institute, Germany) and Felix C Mark (Alfred Wegener Institute, Germany)

Anthropogenic CO_2-emissions continuously add to decreasing seawater pH and enhance the already drastic changes in environmental temperatures. Temperature has a large impact on the velocity of biochemical and enzymatic processes and hence is a key factor defining the performance of ectothermic organisms. Mitochondria are a key element in shaping whole organism energy turnover and functional capacity. This study compared the effects of rising temperature and increased seawater PCO_2 on the energy metabolism between notothenioids from cold-eurytherm (black cod, *Notothenia angustata*), Sub-Antarctic (grey rockcod, *Lepidonotothen squamifrons*) and Antarctic (marbled rockcod, *Notothenia rossii*) waters, and between cephalopods from the Antarctic (*Pareledone* sp.) and temperate (*Eledone cirrhosa*) latitudes. We determined extra- and intracellular blood acid–base parameters, and oxygen consumption at whole animal and mitochondrial level as a measure for routine metabolic rate, in order to compare their abilities for metabolic compensation. Our results showed limited aerobic capacities of high-Antarctic fish mitochondria towards the warmth and higher CO_2-levels. Also the Sub-Antarctic and Austral fish possess only modest capacities to increase their aerobic metabolism under rising temperature and hypercapnia, which are not much higher than those of the Antarctic notothenioids. The mitochondrial responses of cephalopods to an acute temperature rise suggest that they possess similar mitochondrial flexibilities and capacities towards the warmth as fish. Nevertheless, generally more effective capacities for acid–base regulation and larger energy reserves (lipids) in fish compared to cephalopods will putatively make them 'win' the competition for resources over longer time-scales, when seawater temperatures and pCO_2 continue to rise.

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Poster session – Friday 5th July 2013

A5.43

Do future CO_2 levels affect thermal preferendum in temperate marine fishes?

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If fishes are allowed to select a temperature within a gradient, different species and genotypes have different thermal preferenda. Such behavioural temperature choice presumably contributes to ongoing changes in marine fish distributions due to global warming. Oceanic CO_2 levels are predicted to increase as a result of rising anthropogenic CO_2 in the atmosphere. Predicted future CO_2 levels have been shown to influence behaviour of fishes, through effects on GABAergic neurotransmission (Nilsson et al., 2010 *Nature Climate Change)*. GABAergic neurons are implicated in temperature regulation in mammals (Morrison et al., 2008 *Experimental Physiology*), so we investigated effects of future CO_2 on behavioural temperature choice in a temperate marine fish, grey mullet *Mugil cephalus*. Behavioural thermoregulation was investigated in a custom-designed two-chamber shuttle-box system with video-tracking, comparing normocarbia and a CO_2 level of 1.0 mmol l^{-1} (similar to worst-case IPCC scenario in 2100). Mullet were exposed for 96 hours at each CO_2 level and then patterns of temperature choice evaluated over 48 hours. Preferred temperatures did not differ significantly between normocarbia (22.3±1.5°C) and future CO_2 (24.5±2.9°C, n=5 at time of writing). There was evidence, however, that individual mullet selected a wider range of temperatures in future CO_2 (21.1 to 28.2) compared to normocarbia (19.7 to 25.1), indicating that future CO_2 may influence patterns of distribution and abundance in this species.

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Poster session – Friday 5th July 2013

A6 – CHALLENGES TO RESPIRATORY GAS TRANSPORT: A DEDICATION TO GEORGE HUGHES

Organized by: Michael Berenbrink (University of Liverpool) and Scott Mirceta (University of Liverpool)

A6.1
Transport of oxygen and metabolic substrates in migrating birds

Patrick J Butler (University of Birmingham, United Kingdom)

Some birds migrate non-stop for long distances and some also do so at high altitude. Powered (flapping) flight is energetically very costly, and can only be maintained over long periods by the oxidative metabolism of stored substrates. For high altitude migrants, the high demand for oxygen is met in the face of hypoxia and reduced air density, which increases the energy demands of flapping flight. Fat is the most energy dense of the metabolic substrates but is insoluble in water and in mammals its rate of transport across the plasma membrane and into the mitochondria is limited. Nonetheless, birds predominantly use fat during long distance flights, so have evolved effective means of transporting fatty acids from their stores to the mitochondria.
Wild barnacle geese migrate from the high Arctic to southern Scotland and have greater activities of citrate synthase in their flight muscles than captive conspecifics. Heart rate is lower for a given rate of oxygen consumption in wild geese, suggesting that cardiac stroke volume is greater in wild birds. However, wild birds do not exercise more (train) prior to migration. Bar-headed geese migrate over the Himalayas and have many adaptations of their oxygen transport system for high altitude flight. Captive bar-heads are able to run at their maximum sustainable speed, even when breathing 7% oxygen, which produces the equivalent partial pressure to that at the top of Mt Everest. Barnacle geese are not able to do this.

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10:35 Friday 5th July 2013

A6.2
Oxygen limitation at thermal extremes: A comparison across different gas exchange systems

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In aquatic ectotherms a shortage of oxygen has been shown to set thermal tolerance limits, rather than temperature effects *per se*, due to increased energy and hence oxygen demand at high temperatures. This oxygen limitation hypothesis provides one of the few mechanistic concepts to understand and predict the vulnerability of species to the multi stressor effects of climate warming and hypoxia. However, its generality has been challenged. In terrestrial insects, hypoxia does not notably reduce heat tolerance, suggesting that the oxygen limitation hypothesis may not be applicable to tracheated arthropods. Here we remove this apparent discrepancy by comparing thermal tolerance limits in insects from four different orders with different gas exchange systems (tegument, gill and aerial breathing). We show that the oxygen limitation hypothesis is indeed generally applicable, and explain why taxa are differently affected. Aquatic, tracheated ectotherms, which are poor at regulating gas exchange are shown to be especially vulnerable to the multi stressor effects of increased water temperatures and reduced levels of oxygen.

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11:05 Friday 5th July 2013

A6.3
Shape-shifting resolves contradictions in metabolic scaling

David Atkinson (University of Liverpool, United Kingdom) and Andrew G Hirst (Queen Mary University of London, United Kingdom)

In multicellular eukaryotes, metabolic rate generally shows negative allometry (per unit mass, it decreases as body mass increases) within taxonomic groups. Contradicting this is the isometric (directly proportional to body mass) and even positively allometric metabolic scaling seen in morphologically and phylogenetically diverse animals of open water as they develop towards adulthood.
Two prevailing groups of geometry-based hypotheses – one based on size-related limitations of external surface areas, the other on increased challenges of distributing resources and wastes through a larger body – make starkly contrasting predictions about how body shape-shifting affects mass-scaling of metabolic rate. Here, using data on mass-scaling of metabolic rate and body length for 93 and 273 species of pelagic invertebrates, respectively, we resolve contradictions between data and theory.
We show that the average extent of shape change during ontogeny, measured as mass–length exponents, accounts for 66% of the variability in taxon-specific mean respiration–mass scaling exponents. Moreover, the mass–scaling of rarely measured actual surface areas for resource uptake matches metabolic scaling even better than does the scaling of surface area predicted from simple Euclidean geometry, implying that fractal dimension of the uptake surface increases during ontogeny. We thus demonstrate a strong association between elaborated external surface area, but not predicted internal resource delivery, and the distinctively high yet variable metabolic scaling exponents of pelagic invertebrates.
These findings underpin a new explanation for occurrences of near-isometric metabolic scaling in nature, and widespread mixed scaling during ontogeny.

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11:25 Friday 5th July 2013

A6.4

Functional morphology of ventilation in four species of sculpins (Cottoidei)

Stacy C Farina (Cornell University, United States)

Among teleost fishes, substantial diversity in cranial morphology results in variation in gill ventilatory morphologies with functional and ecological consequences. The groundbreaking work of GM Hughes established the basic biomechanics of gill ventilation in fishes and explored variation in gill ventilatory function broadly across teleosts. However, there is still considerable variation in form and function to investigate, especially among closely related taxa. We link size of the branchiostegal apparatus with differences in ventilatory function among four Cottoidei species.

Branchiostegal rays are long, thin dermal bones that articulate with ventral elements of the hyoid arch and form the floor of the gill chamber, contributing to the driving of ventilatory current. We collected functional (kinematic and pressure recordings) and anatomical data from three individuals of each of four species (*Leptocottus armatus*, *Myoxocephalus polyacanthocephalus*, *Hemilepidotus hemilepidotus*, and *Dasycottus setiger*).

Relative size of the branchiostegal apparatus correlates with both ventilation rate ($r=-0.78$, $p=0.003$) and the relative duration of the inspiration phase ($r=0.73$, $p=0.007$). We also found that the four species showed variation in differential pressure profiles on the scale of previously described differences between pelagic and benthic fishes, even though all four species studied are benthic; functional measurements related to these pressure profiles also significantly correlate with relative size of the branchiostegal apparatus.

Quantifying the morphology and functional role of the branchiostegal apparatus can lead to better understanding variation in gill ventilatory mechanics among teleosts.

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11:45 Friday 5th July 2013

A6.5

Air-breathing in the arctic: Influence of temperature and hypoxia on respiration in the Alaska blackfish *Dallia pectoralis*

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The Alaska blackfish is to our knowledge the only air-breathing Arctic fish. It is native to rivers and lakes of Alaska, where hypoxia can occur during both summer and winter. Its oesophagus is partially modified for air-breathing, but since it is trapped under the ice for long periods of the year it likely needs to achieve relatively high rates of oxygen uptake (MO_2) over the gills.

In this study, we measured the partitioning of MO_2 between air and water for 5 and 15°C-acclimated fish in normoxia and hypoxia (25 mmHg), both with and without air access. In normoxia, air-breathing accounted for 10% of MO_2 at 5°C and 23% at 15°C. In hypoxia, air-breathing accounted for 65% of MO_2 at both 5 and 15°C, and the resting MO_2 was not depressed in hypoxia. While 15°C fish lost equilibrium after ~2 hours in hypoxia without air access, 5°C fish lasted at least 12 hours. At both temperatures, however, MO_2 was significantly depressed by 25% in hypoxia without air. Furthermore, the critical pO_2 without air access was not significantly different between acclimation temperatures (pO_2=40 mmHg), indicating low hypoxia tolerance. Aerobic scope measurements with access to air indicated that the fish have the ability to be active in the cold. MO_2 could be increased 4- to 10-fold at both temperatures.

Taken together, the data suggest that the blackfish, rather than being hypoxia tolerant, needs to avoid aquatic hypoxia unless it has access to air.

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12:00 Friday 5th July 2013

A6.6

Gasping for air: Alternative strategies to maintain oxygen delivery during hypoxia and air exposure in an amphibious fish

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Breathing O_2-rich air is advantageous only if air breathing organs in amphibious fishes are in a state of inherent readiness, without a significant latent capability. If not, air exposure may immediately result in hypoxemia and induce a cellular hypoxia response. We tested the hypothesis that acclimation to hypoxia or air involves the same suite of responses that together enhance blood–O_2 transport in amphibious fishes. Using the cutaneous-breathing, mangrove (*Rivulus*) *Kryptolebias marmoratus* that remain active out of water for weeks, we compared fish acclimated for 7 days to normoxic water (control), aquatic hypoxia (~3.5 kPa), normoxic air or aerial hypoxia (~13.5 kPa). Aquatic hypoxia increased blood O_2 carrying capacity through a 30% increase in the haemoglobin (Hb) concentration, while Hb–O_2 affinity (P_{50}) was unaffected. In contrast, air exposure increased Hb–O_2 affinity (decreased P_{50}) by ~25% with no change in erythrocyte numbers or Hb concentration. Aerial hypoxia induced a combination of both responses. Follow up experiments showed that air-exposed fish express Hb isoforms with different isoelectric points relative to control fish in water. These results indicate that O_2 transport is regulated both by O_2 availability and independently by some aspect unique to air exposure, likely CO_2 accumulation. The data refute our hypothesis and suggest that the evolution of terrestrial respiration in vertebrates was not simply the co-option of haematological strategies for aquatic hypoxia, but involved novel adaptations to cope with a very different respiratory media.

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12:15 Friday 5th July 2013

A6.7

The role of oxygen transport in thermal tolerance: From mechanistic aspects to evolutionary innovations

Folco Giomi (Alfred Wegener Institute, Germany) and Hans-Otto Pörtner (Alfred Wegener Institute, Germany)

Temperature and oxygen availability are among the major constraints of complexity and diversity in animal life. More important these two variables interact to shape the boundaries of species performances and fitness playing a leading role in drawing the complex mosaic of animal distribution. Temperature, in particular, shapes the metabolic efficiency of ectotherms since it determines the magnitude of oxygen demand. Thus, the efficiency of oxygen extraction from the environment and its supply to tissues set the breadth and the limits of thermal niche. Within this view the relationship between oxygen transport and thermal tolerance is evaluated in highly eurythermal species and in other taxa radiated in extreme thermal range.

The mechanism that contributes to setting the dimensions of the thermal window in eurytherms has been identified in a model crab evaluating the different elements supporting whole animal oxygen demand. Within the natural temperature range experienced by *Carcinus maenas* the physiological performances, such as oxygen extraction efficiency and cardiac effort, are entirely sustained and remain constant. During acute warming a series of integrated physiological mechanisms extend the range of aerobic performance, and in particular the amount of oxygen bound to haemocyanin is supplied to tissues and fully compensate for the increased demand.

Furthermore, we provide a mechanistic explanation of the repeated evolution of air-breathing in aquatic ectotherms in response to the effects of temperature. We demonstrate that the recurring innovation of aerial transition arises when oxygen transport processes overcome the challenges of extreme heat in aquatic environments.

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12:30 Friday 5th July 2013

A6.8

The contribution of myoglobin to whole-muscle non-bicarbonate buffering in diving carnivores

Scott Mirceta (University of Liverpool, United Kingdom), Jennifer Burns (University of Alaska, United States), Michael Berenbrink (University of Liverpool, United Kingdom)

Metabolism during most mammalian dives remains aerobic in nature. This is achieved through specialised adaptations including localised vasoconstriction which reserves blood oxygen for essential organs. As a result the skeletal muscle beds become isolated and have to rely on their own O_2 stores to fuel their metabolism. In order to maintain efficient respiratory function in isolated tissues, mammalian divers must be capable of buffering acidic conditions, due to both the production of CO_2 during aerobic metabolism and anaerobic end products when muscle oxygen depletes. While several diving mammals show increased whole muscle non-bicarbonate buffering ($b_{muscleNB}$) and Mb content, the contribution of Mb to muscle buffering is generally regarded as negligible (Castellini & Somero, 1981). This study quantifies for the first time the extent to which Mb contributes to $b_{muscleNB}$.

Translated amino acid sequences of 14 carnivore Mbs were newly determined from their cDNA. Acid–base titration of purified Mb allowed development of a model that accurately estimates the specific buffer value (b_{Mb}) for any Mb from its amino acid sequence. We confirm that $b_{muscleNB}$ is significantly increased in diving species and show that b_{Mb} is higher in phocids than in other carnivorans.

Contrary to previous reports, this study reveals that Mb in diving species contributes substantially to muscle buffering, comprising 10–30% of total $b_{muscleNB}$ in diving species compared to 1–2% in terrestrial species. This study provides novel insights into how summative changes to Mb amino acid composition, can have profound adaptive effects on the physiological properties conveyed to the whole animal.

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12:45 Friday 5th July 2013

A6.9

Mechanisms of haemoglobin adaptation in high-altitude vertebrates

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Is it possible to predict which molecular mechanisms are most likely to contribute to biochemical adaptation? Can we predict which mutations – or which types of mutation – are most likely to contribute to adaptive changes in protein function? To address these questions about the inherent predictability of adaptive evolution at the molecular level, I'll present results of recent research on mechanisms of haemoglobin adaptation to high-altitude hypoxia in birds and small mammals. These studies integrate evolutionary analyses of sequence variation with experimental studies of haemoglobin function using site-directed mutagenesis.

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13:40 Friday 5th July 2013

A6.10

Root effect haemoglobins greatly enhance oxygen delivery to the red muscle and gut in teleosts

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The Root effect is a pH-dependent reduction in haemoglobin-O_2 carrying capacity. Unique to ray-finned fishes, the Root effect has been ascribed specialised roles in retinal oxygenation and swimbladder inflation; however, we propose it may also be associated with greatly enhanced O_2 delivery to other tissues, specifically red muscle (RM) and the gut. During a generalized acidosis, catecholamines are released into the blood, activating red blood cell (RBC) Na^+/H^+ exchange (NHE), thus protecting RBC pH and subsequent O_2 binding at the gill. However, plasma-accessible carbonic anhydrase (CA) at the tissues (and absence at the gills) may result in selective short-circuiting of RBC NHE pH regulation within the circulation. When rainbow trout are exposed to elevated water CO_2 (1.5%) red muscle PO_2 increases by 65%, which we estimate could double O_2 delivery with no change in perfusion. Inhibiting plasma accessible CA abolished this effect illustrating its importance to this process. In the gut of marine teleosts, the intestinal lumen secretes bicarbonate to limit Ca^{2+} uptake by carbonate precipitation and aid in water uptake. This is associated with an equimolar transport of H^+ into the blood that is proportional to external salinity. The resulting blood acidification could enhance tissue O_2 delivery by up to 51% with no change in tissue perfusion. Thus, the Root effect in teleosts is associated with greatly enhance O_2 delivery to tissues other than the eye and swim-bladder, which may represent important selection pressures for the evolution of the Root effect.

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14:20 Friday 5th July 2013

A6.11

The role of nitric oxide in the control of breathing of zebrafish (*Danio rerio*)

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In mammals nitric oxide (NO) has a variety of physiological roles related to the hypoxic response including an important role in oxygen sensing. However, nothing is known about the role of NO in the control of breathing in fish. In this study the role of NO in the hypoxic ventilatory response was determined in zebrafish larvae and adults, in combination with immunohistochemistry of neuroepithelial cells (NECs) and calcium imaging of isolated NECs. In larvae, sodium nitroprusside (SNP), a NO donor, caused a dose-dependent increase in ventilation frequency, similar to that caused by hypoxia. However, at higher doses (400 μM) SNP was either inhibitory or excitatory and our data suggest that the ventilatory response to NO represents a balance between chemoreceptor inhibition and central excitation rather than a developmental switch at this stage. Furthermore, exposure of larvae to a nitric oxide synthase (nNOS) inhibitor, 1 mM N-omega-Nitro-L-Arginine Methyl Ester Hydrochloride (L-NAME), blunted the hypoxic ventilatory response. In adults, exposure to SNP inhibited breathing during normoxia or hypoxia. Pre-treatment of fish with L-NAME decreased the hypoventilation response to hyperoxia, and this response was rescued by exposure to SNP. nNOS was not present in adult NECs. Additionally, 400 μM SNP produced a small, but significant increase in intracellular calcium in cultured NECs, consistent with its excitatory effect on K^+ channels and its inhibitory effect on L-type calcium channels observed in mammals. Overall, these results indicate that NO is involved in oxygen sensing and modulates the hypoxic and hyperoxic ventilatory responses in zebrafish.

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14:40 Friday 5th July 2013

A6.12

Hypoxic survival in fishes: How to get to the oxygen and what to do when there is none

Göran E Nilsson (University of Oslo, Norway)

More than most vertebrates, fishes often live in habitats with widely varying oxygen levels. This is a result of the low oxygen content of the water and the slow diffusion of oxygen in water, often combined with a high density of inhabitants. In some cases fishes even have to do without any oxygen from time to time. Some fishes have solved the problem of aquatic hypoxia or anoxia by resorting to air breathing, while others may rebuild their gills to fit the oxygen needs, or have developed high glycolytic capacities with exotic end products. I will present examples of all these strategies from fishes ranging from the cold North to the tropics.

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15:35 Friday 5th July 2013

A6.13

Quantifying the respiratory response of *Paracentrotus lividus* to chronic hypercapnia investigating a carbon capture storage leakage scenario

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Assessing the impact on marine benthos that would arise from localized acidification resulting from a carbon storage leakage scenario is important in order to understand the magnitude of such an event and may also be used as an indication of a leak.
The blood gas and acid–base status of the urchin *Paracentrotus lividus* was determined during chronic hypercapnic exposure of 65 days. The investigation confirmed that *P. lividus* though lacking a significant buffer capacity in the absence of any detectable changes in HCO_3^-, can still tolerate chronic hypercapnia (20,000 ppm) for up to 2 months.
Acid–base disturbances observed in *P. lividus* demonstrated no compensatory change in bicarbonate suggesting a limited short term tolerance and it is predicted that any further elevation in temperature or pCO_2 would be lethal as a result. SEM images of urchin spines indicate reduced growth and ability to regenerate under chronic acidification; furthermore, substantial spine dissolution was identified in *P. lividus* exposure to pH <6.52.
The current investigation highlights the ability of *P. lividus* to tolerate chronic acidification during a two-month exposure; however the respiratory capacity is reduced indicating metabolic depression. It is concluded that increased duration of acidification would lead to high rates of mortality.

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15:55 Friday 5th July 2013

A6.14

Blue blood on ice: How the cephalopod blood pigment haemocyanin helped to colonize extreme habitats

Michael Oellermann (Alfred Wegener Institute for Polar and Marine Research [AWIPMR], Germany), Hans O Pörtner (AWIPMR, Germany) and Felix C Mark (AWIPMR, Germany)

The octopods' worldwide colonization of diverse thermal habitats from -1.8°C to >30°C relied on the capacity of their circulatory and ventilatory system to supply sufficient oxygen for aerobic metabolism at a given environmental temperature. The blood pigment haemocyanin is crucial to fuel the high-performance metabolism of octopods with oxygen, but suffices this task only within species-specific thermal ranges. We thus aim to identify functional, structural and genetic modifications of haemocyanin that allowed octopods to colonise habitats from warm to freezing temperatures. We compared properties of haemocyanin between Antarctic, temperate and warm-adapted octopods using a newly-extended spectrophotometric-pH recording diffusion chamber setup as well as gene sequencing and native gel electrophoresis to explore links to underlying genetic and structural features. Highly resolved oxygen dissociation curves showed not only lower oxygen affinity for Antarctic haemocyanin but also a 20% higher pH independent venous reserve at habitat temperatures compared to haemocyanin from temperate octopods, indicating functional shifts of Antarctic haemocyanin but incomplete use of its full oxygen transport capacity. Among Antarctic octopods, haemocyanin isoforms were partly electrophoretically distinct and contained fewer negatively charged amino acids than temperate species. Amino acid sequences, native electrophoresis and a so far unnoticed increasing asymmetry of oxygen dissociation curves towards lower pO_2 suggest the presence of two functionally differing isoforms.
We conclude that an altered amino acid composition of haemocyanin subunits or a selective use of functionally-different isoforms modulated oxygen binding properties to sustain oxygen transport and thus survival of octopods at sub-zero temperatures.

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16:10 Friday 5th July 2013

A6.15

Neuroglobin in the icefish: A natural knockout for haemoglobin and myoglobin

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Neuroglobin, recently discovered in vertebrates, can bind oxygen and NO. Although a number of hypotheses have been proposed, its role is uncertain. The finding that Antarctic icefish (family Channicthyidae, suborder Notothenioidei) retain the neuroglobin gene despite the loss of haemoglobin, and of myoglobin in most species, may have important implications in the physiology of the brain.[1,2] A detailed structural and functional analysis of the Antarctic fish neuroglobins was carried out by UV-visible and resonance Raman spectroscopies, molecular dynamics simulations and laser-flash photolysis. Comparison of structural and functional properties suggests that the Antarctic fish neuroglobins most likely preserved and possibly improved the function recently proposed for human neuroglobin in ligand multichemistry. Despite subtle differences, the adaptation of Antarctic fish neuroglobins does not seem to parallel the dramatic adaptation of the oxygen carrying globins, haemoglobin and myoglobin, in the same organisms.[3]
1) Cheng et al Gene 2009; 15; 433(1-2):100-1.
2) Cheng et al IUBMB Life 2009; 61(2):184-8.
3) Giordano et al PLoS One 2012; 7(12):e44508

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16:25 Friday 5th July 2013

A6.16

Tracing the evolution of mammalian breath-hold diving capacity using myoglobin net surface charge and fossil body mass

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Extended breath-hold endurance enables the exploitation of the aquatic niche by numerous mammalian lineages and is accomplished by elevated body oxygen stores and adaptations that promote their economical use. Little, however, is known regarding the molecular and evolutionary underpinnings of the high muscle myoglobin concentration phenotype of divers. We use ancestral sequence reconstruction to trace the evolution of this oxygen-storing protein across a 130-species mammalian phylogeny and reveal an adaptive molecular signature of elevated myoglobin net surface charge in diving species that is mechanistically linked with maximal myoglobin concentration. This discovery, together with allometric considerations, provides insights into the tempo and routes to enhanced dive capacity evolution within the ancestors of each major mammalian aquatic lineage, providing a new perspective on the evolution of this iconic respiratory pigment. Our results also support earlier suggestions of recent amphibious ancestries of echidnas, moles, hyraxes and elephants.

Mirceta et al., *Science* 1234192 [in press]

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16:40 Friday 5th July 2013

A6.17

Resolving blood: High-resolution pH and spectrophotometric measurements of native blood microvolumes advance oxygen dissociation experiments

Michael Oellermann (Alfred Wegener Institute, Germany) and Felix C Mark (Alfred Wegener Institute, Germany)

Oxygen dissociation curves have been widely used to understand oxygen transport in humans, diseases and the diverse blood physiology of numerous species. Despite on-going technological progress and a large variety of methods, available devices still fail to combine measurements of minute volumes of native, un-buffered blood with highly resolved and simultaneous monitoring of pH and blood pigment saturation in the same sample.

We aimed to overcome this methodological shortcoming by equipping a gas diffusion chamber with a broad range fibre optic spectrophotometer and a micro-pH optode. Measurements on 15 µl of native haemolymph from the cephalopod *Octopus pallidus* showed rapid responses of pigment saturation and pH to changing gas mixtures (O_2 CO_2, N_2) and yielded sigmoidal oxygen dissociation curves constructed from 120 to 325 broad range spectra, each resolving 2,048 data points. Absorbance spectra displayed an oxygenation dependent haemocyanin peak at 348 nm and a protein peak at 294 nm that likewise decreased upon deoxygenation. Logistic curve fitting revealed increasing curve asymmetry towards lower oxygen partial pressures.

In this study, we demonstrated that a modified diffusion chamber enables the analysis of microvolumes of un-buffered blood, which facilitates replicated, close to *in vivo* measurements of small blood samples and the investigation of multiple parameters. The strongly enhanced data resolution reveals significant spectral details and previously hidden blood-physiological properties. We conclude the modified diffusion chamber to be highly suitable for experimental biologists who demand high flexibility, detailed insight as well as experimental and biological accuracy unified in a single set up.

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Poster session – Friday 5th July 2013

A6.18

Effects of microcystin-LR (*Microcystis aeruginosa*) on cardiorespiratory parameters of matrinxã (B*rycon amazonicus*) during aquatic hypoxia

Nathan Dias Martins (Federal University of São Carlos, Brazil), João Sarkis Yunes (Federal University of Rio Grande, Brazil), Francisco T Rantin (Federal University of São Carlos, Brazil) and Ana L Kalinin (Federal University of São Carlos, Brazil)

Microcystins (MCs) are toxins commonly found in nature, highly toxic and widely dispersed in aquatic environments. The aim of this study was evaluate the effects of MCs on the cardiorespiratory responses of *Brycon amazonicus*. Were used 16 specimens of matrinxã (Wt=193±17g) divided into: control group (control, n=8)-intraperitoneal injection of 0.5 ml of 0.9% saline; group exposed to MC-LR, (Mcis, n=8)-injection of saline plus lyophilized extract containing MC-LR (100µg/kg^{-1} body weight). After 48 hours, specimens were evaluated by flow-through respirometry during aquatic hypoxia: metabolic rate (VO_2-mLO_2.kg^{-1}.h^{-1}), gill ventilation (V_G-mlH_2O.kg^{-1}.min^{-1}), respiratory frequency (f_R-breaths.min^{-1}), ventilatory tidal volume (VT-mlH_2O.kg$_{-1}$.breath^{-1}), O_2 extraction (EO_2-%), water convection requirement (V_G/VO_2-mlH_2O.mlO_2^{-1}) and heart rate (f_H-bpm).The VO_2 groups, Control (56.9±1.7) and Mcis (58.3±2.5) did not differ significantly. The V_G of Mcis showed higher values from normoxic to $P_{in}O_2$ of 40 mmHg. Maximum values were 2347.8±192.2 and 1634.5±48.7, Control and Mcis respectively. The f_R values of Mcis were higher in all $P_{in}O_2$ except 10 mmHg. The maximum value was 132.7±2.9 Mcis and 117.0±1.5 Control. The V_T was not significantly different between groups. The O_2 extraction was lower in Mcis during PinO_2 of 140/60 mmHg, increasing only during 10 mmHg. V_G/VO_2 was higher in Mcis between 100 and 80 mmHg, and also in 10 mmHg, maximum value was 114.5±6,2 Mcis and 93.0±5.0 Control. There was no difference between the fH of these groups. It was concluded that *Brycon amazonicus* used physiological responses that were able to maintain their metabolic rate constant, compensating the effects of MC-LR on their tissues.

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Poster session – Friday 5th July 2013

A6.19

Acclimation to aerial exposure in the long-jawed mudsucker (*Gillichthys mirabilis*)

Corey Jew (Scripps Institution of Oceanography, United States), Andrew Y Gracey (University of Southern California, United States) and Martin Tresguerres (Scripps Institution of Oceanography, United States)

The long-jawed mudsucker (*Gillichthys mirabilis*) is an intertidal wetland goby that frequently experiences aquatic hypoxia and, in response, has been known to gulp air in water or emerge onto land. *G. mirabilis* is however limited in its terrestrial capacity, having a quiescent behaviour out of water and eliciting a similar transcriptional response as aquatic hypoxia. This study aims to test if and how *G. mirabilis* can acclimate to air-exposure after repeated emersion events.

G. mirabilis was exposed to air for eight hours daily over 20 days while control fish were left submerged. On day 21 both groups experienced a hypoxia challenge of 24 hours of emersion. Muscle and liver were sampled on days 0, 10, 20, and 21 after air exposure and analysed for transcriptomic, metabolomic, and enzymatic activity. The hypoxia-acclimated group demonstrated a muted transcriptional response to the hypoxia challenge compared to that of the control. These results show that acclimation of *G. mirabilis* to air exposure does elicit a response at the transcriptional level that can prepare this fish for further exposure. Activity of citric synthase and lactate dehydrogenase (LDH) did not change during acclimation or between treatments in hypoxia challenged fish. This suggests that *G. mirabilis* may already be enzymatically poised, in terms of the glycolytic pathway, to deal with the eight hours of air exposure used for the acclimation treatment but were not able to quickly increase the enzyme activity over the hypoxia challenge despite up-regulation of LDH encoding genes.

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Poster session – Friday 5th July 2013

A6.20

The role of red blood cell size variation in metabolic rate scaling in vertebrates

Zuzana Starostova (Charles University in Prague, Czech Republic) and Lukas Kratochvil (Charles University in Prague, Czech Republic)

Red blood cells (RBCs) are responsible for oxygen transport. Across species, it was demonstrated that RBC size strongly correlates with the diameter of terminal blood capillaries and that the distance of the average tissue cell to the nearest capillary increases with RBC size. Therefore, the RBC size variance should affect oxygen delivery to each cell in an organism and hence the total metabolic rate of the entire organism. The variation in RBC size within animal lineages should thus affect interspecific metabolic scaling.
We estimated quantitative relationships between RBC size and mass-specific metabolic rate in three vertebrate lineages (mammals, birds and eyelid geckos) and found surprisingly strong negative relationship. This relationship may be caused by the mutual correlation between body mass and RBC size in all three groups. It is thus not clear whether RBC size variation is indeed a causal agent of negative metabolic scaling.
To test whether RBC variation affects metabolic scaling independently on body size increase, we followed ontogenetic allometries in resting metabolic rate and RBC size in five species of eyelid geckos. We found linear relationship between body mass and resting metabolic rate in two species without correlation between body mass and RBC size. On the other hand, three species with significant RBC increase during ontogeny showed curvilinear relationship of metabolic rate on body mass.
Our findings provide evidence that variability in RBC size could be connected with important causal agent responsible for negative allometry of metabolic rate among vertebrates.

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Poster session – Friday 5th July 2013

A6.21

Effects of episodic breathing on gas exchange in anaesthetized turtles

Christian Malte (Aarhus University, Denmark), Tobias Wang (Aarhus University, Denmark) and Hans Malte (Aarhus University, Denmark)

The breathing pattern of many ectothermic vertebrates as well as hibernating and diving endotherms is episodic where bouts of consecutive breaths are interspersed by periods of apnoeas. Using anaesthetised freshwater turtles, a species that normally exhibit this breathing pattern, we investigated whether episodic breathing affect pulmonary gas exchange by comparing episodic breathing to evenly spaced breaths at the same overall mean minute ventilation (VE; 30 ml/min/kg). The evenly spaced breaths were employed until CO_2 excretion and O_2 uptake reached steady state, whereupon we changed to six consecutive bouts of either five or 10 breaths, while not affecting VE, but extending the interval between bouts. Finally, we returned to evenly spaced breaths. During the first breathing bout of the episodic pattern, CO_2 excretion and the respiratory exchange ratio (RER) was reduced, and although these levels gradually returned the original levels, the new steady state for CO_2 excretion and RER were lower than that of both the preceding and the subsequent period of evenly spaced breaths. Despite the apparent CO_2 retention during episodic breathing, arterial PCO_2 had only increased by 1 mmHg by the last two breathing episodes. Neither O_2 uptake, nor heart rate or pulmonary arterial flow changed markedly with breathing patterns. The results suggest that episodic breathing is associated with an intrinsically reduced ability to excrete CO_2, but that this was possibly mitigated by a subsequently reduced CO_2 production (i.e. a reduction in RQ).

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16:40 Friday 5th July 2013

A6.22

The integrative nature of respiratory science and the need for a meeting dedicated to promoting it

Markus Lambertz (Friedrich-Wilhelms-Universität, Germany), Kristina Grommes (Friedrich-Wilhelms-Universität, Germany), Wilfried Klein (Universidade de São Paulo, Brazil) and Steven F Perry (Friedrich-Wilhelms-Universität, Germany)

Although early life evolved in anoxia, aerobic respiration has become almost synonymous with life. Respiration embodies a 'meta-system' that is responsible for supplying energy to all remaining systems (Perry & Burggren, *Integ. & Comp. Biol.* 2007;47:506-509). Regardless of the group of organisms (microbes, plants, animals), the level of organization (cellular, organismic, environmental) or the methods (molecular to paleontological) one studies and uses, if it is the goal to truly understand the underlying respiratory structures and processes in both functional and evolutionary context, one ultimately needs a broad view on the topic. Traditional boundaries between the disciplines and isolated, narrowly focused meetings result in limited exchange of ideas and thereby obstruct this broad objective.
Following the SEB meeting 2014 we will meet for the 3rd International Congress of Respiratory Science in Bad Honnef near Bonn (Germany). Located at the romantic Rhine valley, this venue presents great opportunity to discuss all facets of respiration without any artificial taxonomic or methodological barriers. In addition to a fundamentally transdisciplinary approach, one further aim of the meeting is to promote interdisciplinary respiratory networking. To this end it is part of the concept of the ICRS to provide the attendants an all-in-one venue where all participants are housed and served in the same place where the scientific programme takes place. For more information visit the website at www.respiratory-science.org or this poster, where we can discuss some aspects of the integrative nature of respiratory science and provide further details on the ICRS.

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16:40 Friday 5th July 2013

A7 – WHAT ARE THE LIMITS TO AVIAN ENERGY EXPENDITURE?

Organized by: Jon Green (University of Liverpool), Lewis Halsey (Roehampton University) and John Speakman (University of Aberdeen)

A7.1
Beyond energetics: Avian adjustments to environmental demands

Irene Tieleman (University of Groningen, Netherlands)

Energetic limitations of birds are partly set by environmental conditions, but there is more to these limitations than energetics. Environments differ and constantly change, due to natural processes and human impact. To live in different environments, birds have evolved different sets of physiological, life history and behavioural traits. To cope with changing demands in the course of an annual cycle, birds adjust their physiology depending on annual cycle stage and environmental conditions. Since the prudent parent, the energetics perspective on interactions between bird and environment has been expanded to include other physiological systems. I will synthesize data at the interface of energetics and immune function, collected on larks and stonechats. Our lark work includes study of the evolved responses of physiology and life history in tropical, desert and other environments. Our comparative studies of African, European and Asian stonechats in captivity allow us to unravel genetic and phenotypically plastic components. Together, the stonechats and larks from a wide variety of environments shed light on how birds deal with seasonal and geographic environmental variation.

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10:40 Saturday 6th July 2013

A7.2
The prudent parent meets old age: Constraint and restraint in senescing seabirds

Kyle Elliott (University of Manitoba, Canada), John R Speakman (University of Aberdeen, United Kingdom) and Gary Anderson (University of Manitoba, Canada)

As animals age, they are expected to invest more energy in reproduction as they have fewer subsequent chances to reproduce (the 'restraint' hypothesis). The very oldest animals may also show restraint because even a small increase in energy expended during reproduction may lead to death. Alternatively, both young and very old animals may lack the ability to maintain high levels of energy consumption (the 'constraint' hypothesis), leading to reduced reproductive success. Many studies have observed an increase in reproductive success in long-lived animals with age followed by a reduction at the end of life; fewer studies have examined the proximate mechanisms. We examined metrics of aging in free-living long-lived seabirds (murres [*Uria lomvia*] and kittiwakes [*Rissa tridactyla*]). When birds were stressed via capture and handling, but not exogenously with ACTH, corticosterone – responsible for directing energy away from reproduction following stress – was highest in both young and very old birds. BMR declined linearly with age, suggesting strategic adjustments to reduce the effects of metabolism. In contrast, daily energy expenditure was constant with age while antioxidant capacity was high during middle age, and then dropped off at the end of life (when oxidative stress increased). Hormonal cues led to greater investment in an adult's energy stores over its offspring's energy reserves (restraint hypothesis) at the start of life. At the end of life, both hypotheses were supported; energy expenditure was constrained by senescence.

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11:30 Saturday 6th July 2013

A7.3
Oxidative stress does not limit daily energy expenditure in free-living kittiwakes

Jorg Welcker (Norwegian Polar Institute, Norway), Claus Bech (Norwegian University of Science and Technology (NTNU), Norway), Alexander S Kitaysky (University of Alaska Fairbanks, United States) and John R Speakman (University of Aberdeen, United Kingdom)

Daily energy expenditure (DEE) of free-living animals may be limited by a life-history trade-off based on a direct physiological cost associated with an increased metabolic rate. A potential proximate mechanism for such a limitation of DEE is oxidative stress. It has long been hypothesized that the production of reactive oxygen species, inevitable by-products of energy metabolism, may increase with increasing metabolic rate leading to somatic damage and consequently to accelerated senescence and increased mortality. We tested this hypothesis in a long-lived avian model species, the kittiwake, during a period of peak energy demands (reproduction). In two separate experiments we increased parental workload by clipping primary feathers and by brood size manipulation, respectively, and measured metabolic rates, oxidative damage and telomere dynamics. Experimental treatments had only minor effects on DEE indicating that birds were unable or reluctant to adjust overall energy expenditure. However, birds with increased workload decreased their basal metabolism (BMR) thereby increasing their metabolic scope and the amount of energy that could be allocated to reproduction. While a link between workload, oxidative damage and telomere attrition was partly supported by our data, oxidative damage was independent of metabolic rates. These results suggest that breeding kittiwakes down-regulate their BMR when DEE is constrained and add to accumulating evidence that this constraint is unlikely to be related to oxidative stress.

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11:55 Saturday 6th July 2013

A7.4
Why bigger breasts are not always best: The energetics of differential load bearing in birds

Jonathan R Codd (University of Manchester, United Kingdom)

The mechanics of breathing in birds is generally described as centring on the dorsoventral movements of the sternum during inspiration and expiration. However, the sternum is the site of the largest muscle mass used during flight – the pectoralis – which must be moved up and down during each breath. Our recent research has focussed on understanding the importance of moving this large mass during respiration and the impact on bird behaviour. By examining the energetics of breathing in different birds during sitting and standing we are able to examine the significance of moving this large muscle mass during respiration for overall daily energy budgets of birds. This research also has implications for domesticated bird species that are commonly selected for differentially larger breast muscle mass that has been artificially selected for consumption.

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12:20 Saturday 6th July 2013

A7.5
Upper limits to hummingbird metabolic rates

Raul K Suarez (University of California, United States)

Maximum metabolic rates during exercise can be measured as time-averaged values over a prolonged period, aerobic values achieved under conditions wherein rates of mitochondrial ATP synthesis match rates of hydrolysis, or 'anaerobic', unsustainable rates achieved for brief periods when utilization rates exceed rates of mitochondrial ATP synthesis. There can be proximate, mechanistic explanations for ceilings to these rates as well as ultimate explanations.
As body size declines across bird species, wingbeat frequencies increase, resulting in an inverse relationship between body mass and mass-specific metabolic rate. Such allometry in hovering metabolic rate is observed among hummingbirds. Adopting a Rumsfeldian approach, this lecture shall cover 'known knowns' concerning the relationships between functional capacities and metabolic requirements during flight in hummingbirds.
The ceiling to 'burst' exercise in hummingbirds may be imposed by biomechanical limitations. The ceiling to maximum sustained metabolic rates, achieved at low ambient temperature when dietary energy intake rates equal rates of energy expenditure, may be determined, at least in part, by the kidneys.
'Known unknowns', the bases for thesis proposals and grant applications, emerge from discussion of 'known knowns'. However, history demonstrates that openness to the possibility of 'unknown unknowns' can lead to scientific advances, paradigm shifts and great benefits to society.

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13:40 Saturday 6th July 2013

A7.6
When body temperature is increasing: Potential constraints to work rate

Jan-Ake Nilsson (Lund University, Sweden) and Andreas Nord (Lund University, Sweden)

Birds inhabiting hot and dry environments are faced with a problem to get rid of excess heat produced during work. This is further aggravated when water is in short supply, which puts limit on evaporative cooling. It will be reported that birds in such situations are not defending strict homoeothermy but allow their body temperature to increase in pace with ambient temperature. However, birds in much less extreme environments alose invoke above normal body temperatures.
By manipulating brood size in marsh tits (*Poecile palustris*) breeding at temperate latitudes, we aimed to study the extent to which parental effort might affect their possibility to stay homoeothermic. We found pronounced hyperthermia in response to increased parental effort, thus in our case work rate when feeding nestlings. The extent of hyperthermia was also, like in hot environments, modulated by ambient temperatures. Thus, it seems as when the gradient between body and environment is decreasing and lots of heat is produced in the muscles, these birds have trouble getting rid of excess heat.
We speculate to what degree this accumulating heat may put constraints to the work rate of birds. Both of these examples highlight the plasticity in the regulation of avian body temperature, in both cases making it possible to retain heat during periods when the dissipation of excess heat in the body is slow.

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14:30 Saturday 6th July 2013

A7.7
Tinamous, primitive birds with small hearts: Metabolic and cardiovascular limitations

Jordi Altimiras (Linköpings universitet, Sweden), Lina Maria Giraldo Deck (Universidad Mayor de San Andrés, Bolivia), Isa Lindgren (Linköpings universitet, Sweden), Alberto Matthei (Tinamou Chile, Chile) and Alvaro Garitano-Zavala (Universidad Mayor de San Andrés, Bolivia)

Tinamous are ancestral birds (clade: Palaeognathae) together with ratites (ostriches and related species). Scant old data show that tinamous have the smallest heart among birds and this is expected to limit aerobic scope. Our aim was to characterize heart morphometry and to measure metabolic and cardiovascular function in two species of the genus *Nothoprocta*, the Ornate Tinamou (OT), a highland species and the Chilean Tinamou (CT), a lowland species. Relative heart size was 0.24% for OT and 0.28% for CT, significantly smaller than high and lowland chickens (0.54% and 0.42% respectively). Resting metabolic rate is 31% lower in OT than in highland chickens. When exhausted, OT and CT had elevated glucose and lactate levels suggesting a severe oxygen debt, and OT also showed limited capacity to maintain body temperature with a significant and persistent drop in body temperature (over two hours) after the exhaustive bout. Heart rate running on a treadmill at 3 km h^{-1} was 5% lower in OT, indicating that OT cannot compensate for the reduction in heart size with a faster heart rate. In anaesthetised birds, cardiac output and stroke volume of OT was 40% of the cardiac output of chickens in identical conditions. Blood pressure was significantly higher in OT. Altogether, we provide evidence that heart size is a phylogenetically conserved trait among tinamous and that OT cannot compensate aerobically for its small heart. Instead, it relies on anaerobic metabolism incurring in a large oxygen debt while exhausted.
Supported by career grant from LiU to JA.

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14:55 Saturday 6th July 2013

A7.8
Increasing cold tolerance does not elevate oxidative stress in free-living black-capped chickadees wintering in eastern Canada

Magali Petit (Université du Québec à Rimouski, Canada) and François Vézina (Université du Québec à Rimouski, Canada)

In winter, resident bird species living at northern latitudes increase their metabolic rate to cope with cold weather conditions. Several studies showed that animals increasing oxygen consumption (e.g. sudden activity; cold stress) also generate more reactive oxygen species (ROS) that are deleterious for the organism. However, animals are also able to adjust their antioxidant (AO) mechanism to balance ROS production and therefore maintain a constant level of oxidative stress. In this study, we tested (1) whether free-living birds facing harsh winter conditions experienced an increase in ROS production and (2) whether they increased their AO level in order to maintain a constant level of oxidative stress. We captured 175 free-living black-capped chickadees (*Poecile atricapillus*) over two consecutive winters and measured the minimal maintenance costs (BMR), the maximal thermogenic capacity (MSUM) as well as the ROS and AO production. As expected, wintering chickadees increased their BMR (6%) and MSUM (32%) during the winter. We observed positive relationships between MSUM and ROS and AO levels, but not with oxidative stress. Our findings thus suggest that: (1) the increase in cold tolerance in small wintering passerines is associated with elevated ROS production; but (2) that these birds are able to counterbalance this effect by increasing their AO production and maintain a stable oxidative stress level throughout the cold season. Up-regulation of AO defence could therefore be part of seasonal cold acclimatization.

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15:35 Saturday 6th July 2013

A7.9

Oxidative stress and telomere shortening as a potential limitation for metabolic rate

Johan Nilsson (Lund University, Sweden) and Jan-Åke Nilsson (Lund University, Sweden)

Reactive oxygen species (ROS) are highly reactive molecules, mainly produced during the normal cellular metabolism. These molecules can cause considerable damage to the cell by attacking important biomolecules such as DNA, protein and lipids. Under normal conditions, the negative effects of ROS are balanced by antioxidants. Should however the antioxidants fail to neutralize all the produced ROS, the individual might be exposed to oxidative stress. Since most of the ROS are produced during the cellular metabolism, it has been hypothesised that metabolic rate might be limited by how well the individual can cope with oxidative stress.
Here we explore if oxidative status and telomere dynamics are linked to metabolism in wild marsh tits (*Poecile palustris*). We show that nestlings reared in enlarged broods have lower defences against oxidative stress, compared to those reared in reduced broods. We also show that metabolism is linked to antioxidant levels and that a high metabolic rate seems to deplete antioxidants. This finding supports the idea that ROS production and antioxidant status can be an important limitation for individual metabolisms.

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15:55 Saturday 6th July 2013

A7.10

High flight costs, but low dive costs, in auks support the biomechanical hypothesis for flightlessness in penguins

Kyle H Elliott (University of Manitoba, Canada), Robert Ricklefs (University of Missouri-St Louis, United States), Tony Gaston (Environment Canada, Canada), John R Speakman (University of Aberdeen, United Kingdom), Scott A Hatch (Institute for Seabird Research and Conservation, United States) and Gail K Davoren (University of Manitoba, Canada)

Flight is a key adaptive trait. Despite its advantages, flight has been lost in several groups of birds, notably among seabirds, where flightlessness has evolved independently in at least five lineages. One hypothesis for the loss of flight among seabirds is that animals moving between different media face tradeoffs between maximizing function in one medium relative to the other. In particular, biomechanical models of energy costs during flying and diving suggest that a wing designed for optimal diving performance should lead to enormous energy costs when flying in air.
Costs of flying and diving have been measured in free-living animals only in species that use their wings to fly or to propel their dives, but not in animals that do both. Animals that both fly and dive might approach the functional boundary between flight and non-flight. We show that flight costs for thick-billed murres (*Uria lomvia*) and pelagic cormorants (*Phalacrocorax pelagicus*) are the highest recorded for a vertebrate. Dive costs are high for foot-propelled cormorants and low for wing-propelled murres, but still higher than for flightless wing-propelled diving birds (penguins). For murres, flight costs were higher than predicted from biomechanical modelling, and oxygen consumption rate during dives decreased with depth at a faster rate than estimated biomechanical costs.
These results strongly support the hypothesis that function constrains form in diving birds, and that optimizing wing shape for wing-propelled diving leads to such high flight costs that flying ceases to be an option in larger wing-propelled diving seabirds, including penguins.

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Poster session – Friday 5th July 2013

A8: REMODELLING OF PHYSIOLOGICAL SYSTEMS IN RESPONSE TO ENVIRONMENTAL CHANGE

Organized by: Todd Gillis (University of Guelph) and Holly Shiels (University of Manchester)

A8.1
Seasonal remodelling of the host–gut microbe symbiosis in hibernators

Hannah V Carey (University of Wisconsin-Madison, United States)

Animal-associated microbes have profound and diverse effects on animal development, physiology and behaviour. Vertebrate guts are home to the majority of these microbes that number in the trillions and have evolved complex, mutualistic relationships with their hosts. Because host diet is a major factor that influences the structure of microbial communities, seasonal changes in host food type and abundance have the potential to affect microbial communities and alter the host–gut microbe relationship.

To meet metabolic needs, gut microbes degrade dietary components that have escaped small intestinal digestion and absorption. Host-derived substrates such mucin glycans can also be metabolized by certain taxa. Our studies examine the role of the microbiota in gut structure and function in seasonal hibernators that cease feeding throughout the five to six-month hibernation season.

In 13-lined ground squirrels, the luminal and mucosa-associated microbiotas are restructured over the annual cycle, reflecting shifts in abundance of bacterial groups that rely to varying degrees on dietary *versus* host-derived substrates. Phylogenetic diversity is lowest and highest in microbiotas of late winter and spring squirrels, respectively. The intestinal immune system, the primary sensor of gut microbes and their metabolites, is remodelled during hibernation in a manner suggestive of altered communication and tolerance between the host and its microbes. Expression of several enterocyte proteins that modulate the intestinal barrier is also elevated during hibernation.

These studies emphasize the importance of incorporating the potential contributions of gut microbes to remodelling of physiological systems in response to environmental change.

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11:00 Wednesday 3rd July 2013

A8.2
Hibernation: A rhythm of damage and repair?

Robert H Henning (University Medical Center Groningen, Netherlands)

Hibernation represents the most extreme change in physiology in mammals, particularly in small animals. Hibernation consists of periods with a 95–99% decrease in metabolism (called 'torpor') that result in large reductions in heart and respiratory rate, and body temperature. Torpor bouts are interspersed by much shorter arousal periods during which metabolism and animal physiology is normalized.

Remarkably, the repetitive cycles of cooling and rewarming during hibernation do not invoke any signs of organ injury post-hibernation. Nevertheless, closer examination of tissues at various stages during the hibernation cycle reveals particular changes in the expression of specific proteins or in tissue architecture that closely resemble changes in human disease, e.g. lung tissue of torpid hamster shows increased deposition of collagen and increased expression of α-smooth muscle actin, thus resembling human asthma. Importantly, such changes are rapidly normalized during arousal. Thus, hibernation not only features ongoing tissue remodelling during torpor, but also full restoration during arousals.

During my presentation I will address various aspects of remodelling during hibernation in different organs of deep and daily hibernators. Further, I will discuss whether remodelling during hibernation represents tissue damage, the role of body temperature and the molecular mechanism involved, including the role of the cystathionine-β-synthase/H_2S pathway.

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11:40 Wednesday 3rd July 2013

A8.3
Cellular and molecular remodelling of the freshwater turtle heart to low temperature and the heart of a tropical coral reef fish to elevated temperature

Jonathan AW Stecyk (University of Alaska Anchorage, United States)

For ectothermic vertebrates, ambient temperature has been termed the 'ecological master factor' because of its influence on chemical reactions and therefore biological processes. When exposed to a change in temperature, ectotherms can display an array of responses for a particular physiological process, ranging from no compensation against the change, compensation against the change, or augmentation of the physiological response, termed inverse-thermal compensation. My presentation will highlight recent discoveries on how the heart of two species, the freshwater turtle (*Trachemys scripta*) and a tropical coral reef fish (*Acanthochromis polyacanthus*), is remodelled at the tissue, cellular and molecular level in response to decreased and elevated temperature, respectively.

For the turtle, reduced cardiac activity with cold acclimation is accompanied by modifications of gene expression that presumably precondition the heart for winter anoxia. In particular, cold acclimation results in three- to six-fold increases of the mRNA expression of heat-shock protein 90 and heat-shock cognate 70, as well as a remodelling of pacemaker channel gene expression.

Studies on the functional response of the heart of a number of tropical coral reef fishes to climate-change-relevant increases in temperature indicates that cardiac insufficiency contributes to the collapse of aerobic scope that occurs with acute exposure to high temperature. Nevertheless, acclimation studies on *A. polyacanthus* revealed that compensatory changes in maximum oxygen uptake and maximum intrinsic heart rate occur within a short time period.

Like for the turtle heart, changes in cardiac function are reflected by changes in the gene expression of key cardiac contractile components.

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12:10 Wednesday 3rd July 2013

A8.4

Organismal thermotolerance and the cellular stress response in Antarctic fishes: From genomics to protein-level processes

Bradley A Buckley (Portland State University, United States)

The fish fauna of the Southern Ocean possess some of the lowest upper lethal thermal thresholds of any species and inhabit waters that have been near-freezing for millions of years. Evolution in a stable, frigid environment has profoundly affected the physiologies in these species, giving rise to novel adaptations to cold temperatures and dramatically altering other cellular functions and organismal processes.

Despite lacking a traditional heat shock response (HSR), characterized by the induction of heat shock proteins during thermal stress, cDNA microarray analysis reveals that hundreds of genes related to numerous cellular processes are potentially stress-inducible in Antarctic fishes. We propose that a modified version of the broadly conserved cellular stress response (CSR) may exist in cold-adapted species. It is possible that in the absence of the ability to increase molecular chaperoning capacity via the inducible HSR, the CSR in cold-adapted species may favour cell cycle arrest and/or apoptosis.

To characterize the CSR in fishes from the Southern Ocean, we measured the heat-induced production of a key regulator of cell cycle arrest and apoptosis, CCAAT/Enhancer-Binding Protein Delta (C/EBP-δ) in common fish species from McMurdo Sound in the southern Ross Sea. We investigated the effect of heat stress on the expression of growth arrest and DNA damage protein 45 (GADD45), which is involved in cell fate determination, and measured apoptotic events through flow cytometry. The overall goal of these efforts is to build an integrated understanding of the CSR, from genomic responses to cellular events, in environmentally sensitive Antarctic fishes.

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12:40 Wednesday 3rd July 2013

A8.5

Are there coherent patterns in thermal acclimation responses of ectotherms?

Susana S Clusella-Trullas (Stellenbosch University, South Africa)

Understanding the direction and magnitude to which temperature performance parameters of ectotherms can shift through plasticity is essential for assessing the persistence of organisms to environmental change. Yet, general interspecific patterns of these responses are lacking despite their urgent need for predictions of species' vulnerability to climate change.

While it has been suggested that tropical species which live in more stable environments tend to have less plasticity than temperate species occupying more variable environments, support for these patterns has not been forthcoming to date. Importantly, tests for such patterns do not yield congruent results, especially across taxonomic groups and geographic scales.

Here, I use several investigations from my current research to illustrate the lack of coherence in patterns of reversible plasticity (acclimation and acclimatization) of thermal optima and tolerances in terrestrial and marine species, at regional and global scales. This synthetic overview indicates that attempting to identify patterns in acclimation responses of organisms in relation to their thermal habitat will likely require larger datasets than those currently available, more rigorously controlled methodologies and the incorporation of several scales of investigation (e.g. from individuals to populations and species; temporal and spatial) and approaches (molecular and whole-organism).

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12:55 Wednesday 3rd July 2013

A8.6

Environmental remodelling of GABAergic and glutamatergic neurotransmission gave rise to the anoxia-tolerant turtle brain

Les Buck (University of Toronto, Canada)

Earth's changing environment has been a major evolutionary force shaping the diversity of species both in the past and present. Climate cooling over the past 100,000 years has resulted in seasonal ice cover at northern and southern latitudes that has selected for hypoxia and anoxia tolerance in some species, such as freshwater turtles. At the northern reaches of their range North American freshwater turtles spend 4 months or more buried in the mud bottom of ice covered lakes and ponds. From a comparative perspective this gives us the opportunity to understand how a brain, an organ extremely sensitive to reduced oxygen availability in mammals, can function without oxygen for long periods.

Brain function is based on complex inhibitory (off) and excitatory (on) circuits involving the major neurotransmitters gamma-aminobutyric acid (GABA) and glutamate, respectively. When a mammal brain becomes anoxic glutamate levels rise within minutes and results in excitotoxic cell death; this does not occur in anoxic turtle brain. The response in turtle brain has been remodelled – GABA levels rise and I will show that this results in large inhibitory GABA receptor currents and inhibition of glutamate receptor function that together depress neuronal activity. Furthermore, with the onset of anoxia reactive oxygen species (ROS) levels decrease dramatically in an *in vitro* turtle brain sheet and scavenging ROS during normoxia results in a similar rise in GABA receptor and decrease in glutamate receptor currents. Therefore, decreasing ROS levels is a potential low oxygen signal.

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14:00 Wednesday 3rd July 2013

A8.7

Making NO out of thin air

Martin Feelisch (University of Southampton, United Kingdom)

About 20% of the UK population will end up in intensive care at some stage of their life, and more than a third of those will not leave the hospital alive. Cellular hypoxia and systemic inflammation are a near-ubiquitous pathophysiological challenge in this setting, and many important treatment decisions are guided by limited 'hard' data. Reasons for inter-individual differences in outcome remain largely unclear, patient stratification is mostly based on simple physiological read-outs, and supplementary oxygen is given frequently to treat conditions associated with low peripheral O_2 saturation and reduced arterial pO_2.

Hypoxic signalling and inflammation are transcriptionally linked and under tight redox control, which itself is governed by the production/availability of nitric oxide (NO) and reactive oxygen species (ROS). While these processes are well characterized in cellular systems, much less is understood at the whole organism level. This presentation will focus on results from an experimental approach that employs a combination of whole-body physiology and multi-biomarker research while exposing healthy human volunteers to increasing levels of environmental hypoxia at high altitude.

Results from these studies suggest that an adequate production and/or availability of NO is crucial for the ability of humans to tolerate a reduction in oxygen availability. Preliminary systems analysis of data from those studies confirms the feasibility of predicting outcome, i.e. the ability to perform work under hypoxic conditions, by relatively simple measurements of 8–10 plasma read-outs. Our results further suggest that NO-enhancing strategies rather than O_2 supplementation may improve survival under conditions of reduced O_2 availability.

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14:40 Wednesday 3rd July 2013

A8.8
The effects of acute anoxia and reoxygenation on trout and turtle cardiomyocyte function

Gina L Galli (University of Manchester, United Kingdom), Mark Scott (University of British Columbia, Canada) and Jeffrey Richards (University of British Columbia, Canada)

The heart of the freshwater turtle, *Trachemys scripta*, is remarkably tolerant to anoxia/reoxygenation. It is not known whether anoxia tolerance extends to the isolated turtle cardiomyocyte, and whether physiological adaptations exist to allow cellular function without oxygen. To this end, we subjected isolated turtle cardiomyocytes to acute anoxia/reoxygenation while measuring contractility, intracellular Ca^{2+} and pH. As a control, parallel experiments were run on cardiomyocytes from an anoxic-intolerant species, the rainbow trout. Ca^{2+} and pH were measured with epi-fluorescent microscopy using the fluorescent indicators Fura-2 and BCECF, respectively.
Turtle and trout myocytes exposed to 20 minutes of anoxia exhibited a sharp decline in pH from 7.4 to 6.2 and a rise in diastolic and systolic Ca^{2+}. The magnitude of contraction decreased in both species during anoxia, suggesting myofillament Ca^{2+} sensitivity declined, but this effect was more pronounced in trout cardiomyocytes (~65% reduction) compared with turtle (~25% reduction). In both species, 20 minutes of reoxygenation led to a recovery of intracellular Ca^{2+} to pre-anoxic levels, while pH increased to 7.7 and the magnitude of contraction recovered to approximately 75% of pre-anoxic values.
Results from this study suggest the anoxia tolerance of the turtle heart extends to the level of the cardiomyocyte, and an ability to defend myofillament Ca^{2+} sensitivity may protect the cardiomyocyte against anoxic damage.

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15:10 Wednesday 3rd July 2013

A8.9
Muscle metabolic remodelling in high altitude native mice

Grant McClelland (McMaster University, Canada)

Small mammals living in alpine environments must deal with combined low O_2 and temperature. High altitude (HA) adaptations for the efficient use of O_2 may shape skeletal muscles for effective locomotion, but thermogenic adaptations may favour heat production over useful work. How these stressors may interact to affect genetic adaptation, and the importance of phenotypic plasticity on muscle metabolism are unclear.
We show intra- and interspecific increases in exercise carbohydrate use and thermogenic lipid use in HA Andean and deer mice, respectively. These differences were not associated with large differences in muscle capacity for carbohydrate use in Andean mice, but HA deer mice show higher capacities for muscle fatty acid use and aerobic metabolism. This capacity was reduced in muscles of F_1 decedents of HA deer mice, but partially restored with hypoxia acclimation.
These data suggest muscle metabolic remodelling differs between genera, and that developmental and phenotypic plasticity contribute to muscle phenotypes of HA deer mice.

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15:25 Wednesday 3rd July 2013

A8.10
Remodelling of muscle mitochondria in response to environmental change

Christopher D Moyes (Queen's University at Kingston, Canada)

Mitochondrial plasticity is an important component of an animal's ability to respond to bioenergetic challenges. The main 'quantitative' strategy is alteration of mitochondrial content. 'Qualitative' strategies include changing orthologues to alter the structure and function of specific enzymes. Both strategies depend upon control of expression of mitochondrial genes, both nuclear- and mtDNA-encoded. Focusing on fish, my lab explores how acclimation to temperature and oxygen affects the regulation of mitochondrial gene expression. In this talk, I will discuss:
(i) the control of mitochondrial biogenesis in response to cold acclimation, focusing the transcriptional regulation of cytochrome oxidase (COX) genes; and
(ii) the evolutionary variation in COX4 orthologues, which are known, in mammals, to be differentially responsive to hypoxia.
Though many aspects of muscle mitochondrial function are similar across vertebrates, there appear to be fundamental differences in the way fish and mammals coordinate and regulate mitochondria genes.
Supported by NSERC Canada.

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16:15 Wednesday 3rd July 2013

A8.11
Muscle mitochondrial efficiency, the energetic key to survive during winter fast in King penguin chicks

Pierre-Axel Monternier (Ecology of Natural and Man-impacted Hydrosystems, France), Vincent Marmillot (Ecology of Natural and Man-impacted Hydrosystems, France), Jean-Louis Rouanet (Ecology of Natural and Man-impacted Hydrosystems, France) and Damien Roussel (Ecology of Natural and Man-impacted Hydrosystems, France)

King penguins (*Aptenodytes patagonicus*) have exceptionally long breeding cycle with extended chick-rearing periods. During the cold austral winter, king penguin chicks are infrequently fed by their parents. Although king penguin chicks are socially and morphologically well adapted to harsh environmental conditions, they experience a severe energy challenge while fasting in the cold during the sub-Antarctic winter. These energetic constraints led to adaptive responses that primarily trigger a whole range of energy sparing mechanisms.
The aim of the study was to investigate whether mitochondrial plasticity might be a proximal trait involved in some energy sparing mechanisms. As skeletal muscle is the main thermogenic tissue in birds, we explored his possible involvement in metabolic adaptation during fasting.
King penguin chicks were experimentally submitted to a fast/re-fed cycle. At the end of each experimental stage whole body energy expenditure was measured and mitochondrial oxidative phosphorylation efficiency was studied using carbohydrate-derived (pyruvate) or lipid-derived (palmitoyl-carnitine) substrates.
Our results showed that the resting metabolic rate was lower in fasted chicks compared with fed chicks. Interestingly, mitochondrial oxidative phosphorylation efficiency was increased in fasted chicks, suggesting that to produce a given amount of ATP, less oxygen, i.e. less energy was consumed. This increased ATP yield was selectively associated with the use of lipid-derived respiratory substrate.
Our study showed that muscle mitochondria adjusted their efficiency toward thrifty mechanisms. Such subcellular metabolic modification would be a key element to increase the survival of chicks in such an extreme environment regarding the temperature and the very limited energy supply.

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16:45 Wednesday 3rd July 2013

A8.12

Capacity and regulation of thermal acclimation vary between generations in the short-lived mosquitofish (*Gambusia holbrooki*)

Frank Seebacher (University of Sydney, Australia), Julian Beaman (University of Sydney, Australia) and Alexander G Little (University of Sydney, Australia)

Environmental variability can influence population persistence. It is therefore important to understand whether and how animals can compensate for environmental variability, and thereby increase resilience of natural populations. Evolutionary theory predicts that in fluctuating environments selection should favour developmental modifiers that reduce phenotypic expression of genetic variation. The expected result is that phenotypes are buffered from environmental variation across generations. We tested this prediction in the mosquitofish (*Gambusia holbrooki*).

We hypothesized that the spring generation (cool environment) would acclimate by increasing the concentration of regulatory transcription factor mRNA and activities of rate-limiting enzymes (hierarchical regulation) to compensate for the negative thermodynamic effects of lower temperatures on metabolic and locomotor performance. In contrast, the summer-born generation (warm environment) would show less capacity for acclimation and hierarchical regulation.

We show that fish from both generations acclimated, but that there were significant differences in the phenotypic consequences of acclimation. The overall result was that sprint performance, metabolic scope, and the activities of cyctochrome c oxidase and lactate dehydrogenase were buffered from environmental change, and did not differ between spring and summer fish at their natural water temperatures of 15°C and 25°C, respectively. We used metabolic control analysis to show that regulation of metabolic scope and locomotor performance was primarily hierarchical in spring fish, but less so in summer fish. We show that the interaction between developmental and reversible acclimation can render physiological performance of a natural population independent from climate variation.

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17:00 Wednesday 3rd July 2013

A8.13

Phenotypic plasticity of the teleost heart: Changing form and function in response to thermal acclimation

Todd E Gillis (University of Guelph, Canada) and Jordan M Klaiman (University of Guelph, Canada)

Many teleost species are seasonally exposed to more than a 10°C shift in environmental temperature. Such a change in temperature has significant potential to influence the ability of the heart to remain functional due to changes in contractile function. For example, a 10°C drop in temperature causes the mammalian heart to become non-functional due to a loss of Ca^{2+} sensitivity. It is therefore of interest that a number of fish species including the rainbow trout and zebrafish remain active when water temperatures decrease in the winter. In trout, this is possible through extensive remodelling of the heart across multiple levels of biological organization. For example, cold acclimation causes a change in the expression of multiple cardiac genes, a decrease in contractile protein phosphorylation, an increase in cardiac connective tissue content and changes in cardiac morphology. Interestingly, warm acclimation has the reverse effect. These observed changes in the trout heart caused by cold acclimation result in increased contractile function with specific changes including an increase in the activity of actin-myosin ATPase and an increase in the Ca^{2+} sensitivity of skinned cardiac trabelculae. At the level of the whole heart these changes translate into an increase in ventricular developed pressure with no change in chamber stiffness. Maintenance of cardiac function in these fish following thermal acclimation therefore requires an extensive and coordinated remodelling response.

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17:15 Wednesday 3rd July 2013

A8.14

Morphological alteration of seminiferous tubules of testes of Wistar rat offspring exposed to alcohol during pregnancy and/or lactation

Josephat E Onu (Usmanu Danfodiyo University, Nigeria), Bankole O Oke (University of Ibadan, Nigeria), Peter C Ozegbe (University of Ibadan, Nigeria) and Johnson O Oyewale (University of Ibadan, Nigeria)

This paper presents the effects of alcohol on the morphology of seminiferous tubules of testes of Wistar rat offspring exposed to alcohol during pregnancy and/or lactation. Seventy-five adult female Wistar rats divided into three groups of 25 each and their offspring were used. The offspring of group 1 served as control, those of group 2 were exposed to 2 g/kg body weight of 30% ethanol (v/v) during pregnancy and lactation (APL) while those of group 3 were exposed to the same dose of ethanol during lactation only (AL). At Day (D) 7, 14, 21, 35 and 49 of postnatal life, five male offspring were randomly selected from the three groups and sacrificed. After the sacrifice, the two testes were dissected out and then prepared for routine histological evaluation.

The result of the study showed that the seminiferous epithelia of the testes of the alcohol-exposed groups were characterized by few adluminal as well as defoliated germ cells. The result further showed significant reduction ($p<0.05$) in the diameter of the seminiferous tubules of the testes in alcohol-exposed groups. This histomorphometric effects which persisted into adulthood may have implications on the fertility of male offspring of dams who abuse alcohol during pregnancy and/or lactation.

Keywords: Alcohol, pregnancy, lactation, testes, histomorphometry, Wistar rats

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Poster session – Thursday 4th July 2013

A8.15

Morphophysiological remodelling of osmoregulatory system in euryhaline starred sturgeon, *Acipenser stellatus* Pallas, in response to changing environmental salinity

Lyudmila S Krayushkina (St. Petersburg State University, Russia)

The euryhaline species of acipenserids, when exposed to changes in environmental salinity, are capable of maintaining a relative stability in blood serum osmolarity, through a shift between their osmoregulatory patterns (hyperosmotic pattern persists in fresh water, whereas hypo-osmotic – in sea water). This shift is connected with important morphophysiological remodelling of the system of organs that are responsible for supporting the osmotic homeostasis. Such remodelling, as demonstrated by an acclimation of immature starred sturgeon transferred from fresh water to simulated Caspian Sea water (12.5‰), is described in this paper.

In response to salinity influence, the neurophysin-immunopositive (IP) neurosecretory cells of the preoptic nucleus in the hypothalamus and ACTH-IP cells of the proadenohypophysis transfer into the excretory state (in an hour after the start of the experiment). The inter-renal gland reveals an increase of cellular biosynthetic activity and the subsequent excretion of steroid hormones (one hour and 24 hours, accordingly). Further, chloride cells of the branchial epithelium transfer into the excretory state, after their ultrastructural changes and an increase in Na^+/K^+ ATPase activity. The kidney glomeruli shrink; the glomerular filtration, as well as the diuresis, Na^+ reabsorption and Na^+/K^+ ATPase activity are reduced; and the water reabsorption is increased. During the remodelling processes, the observed blood serum osmolarity is increased by 40%, as compared with its level measured in fish from the freshwater environment. In 72 hours, a shift to hypo-osmotic regulation occurs; the blood serum osmolarity goes down and approaches its baseline level.

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Poster session – Thursday 4th July 2013

A8.17

Hypoxia episode at larval stage has a long-term effect on juvenile growth of European sea bass (*Dicentrarchus labrax*)

Gwenaëlle Vanderplancke (Ifremer, France), José-Luis Zambonino-Infante (Ifremer, France), Guy Claireaux (UBO – LEMAR UMR 6539, France) and David Mazurais (Ifremer, France)

Coastal ecosystems which are nursery areas for many marine fish species are more frequently and severely affected by hypoxia episodes over the past 50 years due to global warming and eutrophication. The present study aims at understanding the short- and long-term effects on physiological functions and to evaluate the consequences in term of life-traits of non-lethal hypoxia exposures during the larval period on European sea bass.

Post-hatching larvae were reared in normal oxygen condition during 1 month before being divided in two groups. The 'control' group stood in normal oxygen condition (100% air saturation), while larvae of 'challenged' group were submitted during 8 days to a moderate hypoxia (40% air saturation). We evaluated the short-term impact of hypoxia on larvae by analysing several phenotypic criteria. Thus, we demonstrated that larvae exposed to hypoxia have lower growth and exhibited a shift from aerobic toward anaerobic metabolism. The two groups of fish resulting from this experiment were next kept in similar rearing conditions during 8 months in order to examine the long-term effect of the hypoxia conditioning. At juvenile stage, fish were submitted to a nutritional challenge that revealed lower growth potential in fish having experienced the hypoxia episode during their early life.

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Poster session – Thursday 4th July 2013

A8.18

Physiological and behavioural compensation in lizards depend on thermal history

Helene Basson (Stellenbosch University, South Africa) and Susana Clusella-Trullas (Stellenbosch University, South Africa)

Ectotherms can mitigate environmental variability via behaviour and/or physiology. According to the conceptual model of thermoregulation, thermoregulation is only a viable strategy when the benefits outweigh the costs. This model increases in complexity if the responses of behaviour and physiology are plastic, such that plasticity of physiology and behaviour may either compensate for or compromise the ability to buffer climate variation. We measured the plasticity of preferred body temperature (T_{sel}), resting metabolic rate (RMR) and water-loss rate (WLR) by collecting lizards in either summer or winter and exposing them to ecologically-relevant, short-term temperature treatments ($Temp_{Tr}$: low, average or high temperature regimes for each season).

Results show that winter lizards exposed to different $Temp_{Tr}$ had no plasticity of T_{sel}. By contrast, summer lizards exposed to the high $Temp_{Tr}$ had lower mean T_{sel} than the other two $Temp_{Tr}$ groups. Winter lizards showed plasticity of RMR but there were no plastic responses of RMR in summer-collected lizards. WLR did not respond to short-term $Temp_{Tr}$ in summer or winter. Comparisons between seasons indicated that WLR and T_{sel} were higher in summer than winter, but no differences in RMR were found.

These results suggest that thermal history has a marked impact on behavioural and physiological responses and depending on the time-scale, may have contrasting effects on the traits investigated. Therefore, assessing the magnitude and direction of these plastic responses at different temporal scales is essential for estimating potential compensatory mechanisms of ectotherms facing climate change, with implications for understanding the costs of thermoregulation.

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Poster session – Thursday 4th July 2013

A8.19

PCB-153 and temperature cause restructuring of goldfish membranes: Homeoviscous response to a chemical fluidizer

Alexander C Gonzalez (University of Ottawa, Canada), André Odjél (University of Ottawa, Canada) and Jean-Michel Weber (University of Ottawa, Canada)

Ortho-substituted PCBs intercalate between phospholipids in a manner similar to cholesterol, increasing membrane fluidity. Such fluidization disrupts key membrane proteins like Na/K-ATPase, with far-reaching physiological consequences. Ectotherms have a well-developed homeoviscous response to counter the effects of temperature on membrane fluidity, but how this response is induced remains murky. PCBs provide an experimental tool to investigate whether changing temperature is necessary to induce a homeoviscous response. The membrane composition of gill, white muscle, liver, and brain was measured in goldfish exposed to four treatments in a 2 x 2 factorial design (acclimated to 5 or 20°C, and exposed or not to PCB-153). Because Δ6 and Δ9 desaturases mediate changes in membrane unsaturation, their expression was also measured in gill and liver. We hypothesized that chemical and thermal stress would cause similar adjustments in phospholipid unsaturation, membrane cholesterol, and desaturase expression. PCB-153 caused a homeoviscous response via a decrease in membrane unsaturation (gill) or an increase in cholesterol (brain and liver), but no change in desaturase expression could be detected.

The mechanisms used to respond to chemical and thermal stress were different and varied greatly between tissues. This study is the first to show that *in vivo* chemical stress from a membrane fluidizer can cause a homeoviscous response in ectotherms in the absence of changes in temperature. It suggests that homeoviscous adjustments are triggered by changes in membrane fluidity rather than temperature, and it implies the existence of a molecular sensor of membrane fluidity.

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Poster session – Thursday 4th July 2013

A8.20

The effects of thermal remodelling on the passive properties of the fish heart

Adam N Keen (University of Manchester, United Kingdom) and Holly Shiels (University of Manchester, United Kingdom)

Prolonged exposure to low or high temperature can cause cardiac remodelling in many ectothermic species. The fish provides a well-studied model, where low temperature remodelling has been shown cause significant cardiac hypertrophy, and high temperature to cause suppression of growth or muscle atrophy. In addition to augmentation of electrical conductance and metabolic pathways, cardiac remodelling can alter the passive properties of the heart. Differences in passive properties are primarily caused by structural remodelling of intracellular components and the extracellular matrix. To explore the effects of temperature dependent cardiac remodelling on the passive properties of the heart, we have:

- Generated pressure volume curves to calculate cardiac chamber compliance, distensability and maximum filling volumes.
- Tissue histology was used to quantify histological differences at the tissue level, allowing measurement of differences in cell number and size, as well as densities of extracellular proteins (collagen and elastin).
- Infra-red spectroscopy was used to image tissue, producing colour maps of samples, so that areas of different infra-red spectrums could be distinguished. In addition, spectrums were also used to look for changes in densities of specific compounds (e.g. collagen) and to determine the phosphorylation state of compounds, showing changes in metabolic pathways.

Results will be discussed in relation to mammalian models of hypertrophy.

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Poster session – Thursday 4th July 2013

A8.21

Pre- and postnatal redox status of the rat foetuses exposed to different intrauterine environments: Role in diabetogenic programming

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Maternal health can induce changes in glucose homeostasis pre- and postnatal and leads to type 2 diabetes in the adulthood. We studied pre- and post-natal redox and oxidative status of F_1 offspring of different maternal health challenges and to clarify their role in the diabetogenic programming. Four groups of female Wistar rats were used (diabetic, obese, malnourished and control). Few pregnancies were terminated at gestational day 17 (prenatal samples), the others were completed to term and the offspring were followed up for 30 weeks. Prenatally, redox status of the foetuses of diabetic and obese mothers shifted toward more oxidizing environment which results from elevated oxidative stress and impaired antioxidants as indicated by elevated tissues content of 8-oxo-G and lipid peroxidation. Foetuses of malnourished mothers show mild derangements. Postnatally, impaired glucose tolerance (IGT) and insulin resistance in the offspring of diabetic and obese mothers was detected at 15 weeks of age and at week 30 in the offspring of malnourished mothers. Offspring of diabetic and obese mothers suffers from oxidative stress from the first week of age. The markers of oxidative stress are correlated with the metabolic status. Pancreatic nuclear and mitochondrial DNA contents of 8-oxo-guanine are elevated in the offspring of diabetic mothers pre- and postnatally. Intrauterine environments can induce alterations in the fetal tissues redox status pre- and postnatally which may play an important role in the programming of the metabolic state of the offspring. The authors acknowledge the financial support by Science and Technology Development Fund-Egypt

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Poster session – Thursday 4th July 2013

A8.22

The warming and feeding effects on oxidative balance drive the strategy of resources allocation

Claire Hemmer-Brepson (Irstea – UR HYAX, France), Caroline Romestaing (Université Lyon 1, France), Yann Voituron (Université Lyon 1, France) and Martin Daufresne (Irstea – UR HYAX, France)

Global change has many consequences on ecosystems. Temperature increase and the change in food availability are the drivers receiving the most attention. Both are known to affect the main life history traits of ectotherm species and seem to act on their oxidative physiology. To understand how these environmental changes can impact species, we have set up a 12-month experiment with two contrasted (nonstressful) temperatures and two feeding treatments (*ad libitum* and caloric restricted) on a fish (*Oryzias latipes*). These four groups exhibit different strategies of resources allocation toward growth, maintenance and reproduction as it is suggested by the TSR (temperature-size rule) and due to the thermal and the feeding effects on oxidative balance. Our results show that: (i) individuals reared in warmer conditions present a higher initial growth rate, an advanced maturity and a smaller adult size; (ii) warm-acclimated individuals exhibit a higher metabolism and consequently present more oxidative damage (TBARs and carbonylated proteins) and allocate less energy toward maintenance (SOD, GPx); (iii) due to these less efficient antioxidant capacities, fish from the warmer groups show a higher mortality rate; and (iv) their energy seems to be preferentially allocated to reproduction. Finally, caloric restriction seems to delay deleterious effects in the warmer groups but not in colder ones. This study provides evidence that global change should have consequences on ecosystem via its impacts on individual life history strategies, even with changes considered as non-stressful for a given organism.

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Poster session – Thursday 4th July 2013

A8.23

Thermal tolerance of Atlantic salmon (*Salmo salar*) and the physiological and cellular effects of natural thermal cycles

Suzanne Currie (Mount Allison University, Canada), Melanie Gallant (Mount Allison University, Canada), David Summerby-Murray (Mount Allison University, Canada) and Sacha LeBlanc (Mount Allison University, Canada)

Water temperatures of salmon-rich rivers regularly exceed the fish's putative lethal limit; however, there is little known about the thermal tolerance of fish in these warming waters. Here, we examined the effects of natural thermal cycles on: (1) cellular and physiological markers of stress and recovery; and (2) on the critical thermal maximum (C_{Tmax}) in Atlantic salmon.

Juvenile salmon were exposed to thermal cycles mimicking recent water temperatures from the Miramichi River, New Brunswick, Canada with a maximum temperature of 26°C. We observed a significant increase in HSP70 levels in red blood cells, heart, liver and red and white muscle into recovery from the thermal cycle, with tissue-specific differences in the onset temperature and magnitude of the response. Plasma glucose and lactate significantly but transiently increased at the thermal peak, with no change in activity of the aerobic enzyme marker, citrate synthase in the heart. Liver glycogen plummeted at the peak temperature and remained low throughout the thermal cycle. Red blood cell surface area decreased significantly by the peak of the thermal cycle possibly indicative of the appearance of newer, younger cells. Despite these physiological and cellular responses, there was no significant effect of a natural thermal cycle on the C_{Tmax} of fish; however, condition factor and C_{Tmax} were positively correlated. Overall, a single natural thermal cycle is stressful and metabolically costly for Atlantic salmon with deleterious implications for prolonged exposure to warming and fluctuating temperatures.

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Poster session – Thursday 4th July 2013

A8.24

Comparative transcriptome analysis of *Oreochromis mossambicus* and *O. niloticus* adaptation to fresh or salt water

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Tilapias are a group of fish species (Family: Cichlidae) native to fresh and brackish waters, however, many tilapias are tolerant to high salinity and thrive even in seawater. Differences in salinity tolerance were observed in two tilapiine species, *Oreochromis mossambicus*, which is salinity tolerant and *O. niloticus*, which is more sensitive.

Aiming to characterize the underlying mechanism differing their salinity tolerance we analysed the transcriptomic response of those two species to salt- and freshwater acclimation, in the anterior and posterior intestines. We have sequenced mRNA using the Illumina Hi-Seq technique, analysed gene expression and gene-ontology (GO) patterns, and further characterized sequence variation, and expression patterns in additional organs using qPCR analysis, of several genes that were found to be involved in osmoregulation. Our analyses indicate a different environmental dependent gene expression patterns for each species and intestinal section. Overall, between 182 and 404 genes were significantly up-regulated (p <0.001) with at least 3.5-fold expression increase in either fresh or salt water, in the eight analysed treatments. These genes included various ion and water channels, nutrient transporters, and tight junction proteins. The species comparison resulted with differential expression patterns of specific genes and GOs, including dozens of genes with inversed salinity response between these two species.

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Poster session – Thursday 4th July 2013

A8.25

Effects of body temperature on the senescence: An experimental approach measuring the oxidative stress in the teleost *Notobranchius furzeri*

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Retarding the effects of ageing contributes to increasing quality of life. Many recent studies have investigated the effects of several treatments which could potentially delay senescence and extend life span. Among these, calorie restriction, reduction in body temperature and medicated food were claimed to reduce ageing effects. The effects of these treatments on the senescence delay are measured observing the decline in the efficiency of key biological functions. These effects could be measured at individual, tissue and/or cell levels. At the cell level, oxidative damage is considered to be a major causal factor and consequently a reliable indicator of senescence. Through the assessment of oxidative stress, this study aims to investigate the effect of body temperature on the delay of senescence in a short life span teleost fish *Notobranchius furzeri*. Fish were exposed to two different temperatures and experimented at different periods of their life. Oxidative stress has been investigated measuring the oxidative damage (through lipid peroxidation) and the activity of antioxidant enzymes (catalase and superoxide dismutase) in the liver and axial muscles of fish. These results, and further studies carried out at the individual and tissue levels are integrated in ALIVE (Ageing and functional integrity: modulation by Life-extending treatments in a Vertebrate model with extremely short lifespan) which aims to increase our knowledge concerning the modulation of the ageing-related effects by life extending treatments.

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Poster session – Thursday 4th July 2013

A8.26

Maternal obesity and malnutrition in rats differentially affect glucose sensing in the muscles and adipose tissues in the offspring

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The intrauterine environment, programs to a certain extent the health of an individual throughout life. This effect has been called 'foetal origin of adult disease'. The altered maternal/foetal metabolism appears to be associated with a diabetogenic effect in the adult offspring even in the absence of genetic predisposition.
We aimed to study the effect of different maternal health stats (obesity and malnutrition) on the offspring's muscle and adipose tissues glucose sensing and adipocytokines production to clarify the mechanism of intrauterine programming of diabetes. Also, role of postnatal feeding was assessed. Three groups of female rats were used; obese, malnourished and normal. After pregnancy and delivery the offspring were weaned on control diet or high caloric diet (HCD) and every 5 weeks 5 rats of each group were used for construction of glucose tolerance test and used to obtain tissues.
The results indicated that maternal obesity impair glucose tolerance and imbalanced adipocytokines in the offspring from the 15th week of age even under control diet and the situation is worse under HCD. The offspring of malnourished mothers show normal and even better glucose tolerance under control diet, while those offspring under HCD show impaired glucose sensing and tolerance only at older age than obese group.
Maternal obesity and malnutrition differentially affect glucose sensing and tolerance in the muscles and adipose tissues in the offspring. Postnatal feeding appears to play a central role in these effects.
The authors acknowledge the financial support by Science and Technology Development Fund–Egypt

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Poster session – Thursday 4th July 2013

A8.27

Consequences of constitutive expression of molecular chaperones heat shock proteins 70 and 90: Lessons learned from cold-adapted Antarctic fishes

Marissa Lee (Portland State University, United States) and Bradley A Buckley (Portland State University, United States)

Fishes of the Nototheniidae family, common to the Southern Ocean, dominate the piscine biomass in the coastal waters of McMurdo Sound, Antarctica. These fishes occur below the Antarctic Circumpolar Current, which envelopes the continent, and so have evolved in the near freezing, stable, polar environment for millions of years. Some species of Antarctic fishes have lost the ability to induce the heat shock response during exposure to heat stress. However, heat shock proteins (Hsps) are produced constitutively in these species. Persistent exposure to the extreme cold can cause protein aggregation and therefore, elevated protein folding assistance becomes necessary. Thermal tolerance was characterized for three different co-occurring Antarctic fish congeners. Two potential explanations for inter-specific variation in thermal tolerance were examined. The first was the effect of body mass on thermal tolerance and the second was the role that inter-specific variation in the constitutive expression of the molecular chaperones, Hsp70 and 90, plays in affecting organismal heat tolerance limits. Thermal tolerance varied in the following relative manner: *T. bernacchii* > *T. nicolai* > *T. newnesi* with more massive individuals having longer survivability in some but not all cases. Levels of Hsp70 and Hsp90 vary according to tissue, but do not explain differences in thermotolerance among species. Heat stress fails to evoke a heat shock response upon thermal exposure in these species, with the result of perhaps favouring cell cycle arrest and apoptosis pathways.

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Poster session – Thursday 4th July 2013

A8.28

Compensation capacities for ocean acidification in the Austral Nototheniid *N. angustata*

Felix C Mark (Alfred Wegener Institute, Germany), Anneli Strobel (Alfred Wegener Institute, Germany), Dan W Baker (University of Auckland, New Zealand), Michael Oellermann (Alfred Wegener Institute, Germany), Fathima I Iftikar (University of Auckland, New Zealand), Hans O Pörtner (Alfred Wegener Institute, Germany) and Anthony JR Hickey (University of Auckland, New Zealand)

In this study, we investigated mitochondrial and whole animal tolerance to temperature and hypercapnia in the Austral Nototheniid *Notothenia angustata* as a comparison to its Antarctic congeners. One group of animals was acclimated under hypercapnic conditions (0.2 kPa CO_2, 15 days, n=6), the control group was kept under normocapnic conditions (0.04 kPa CO_2, n=10). Permeabilized heart mitochondria of both groups were assayed in normocapnic (0.04 kPa CO_2) and hypercapnic (3.0 kPa CO_2) respiration buffers at 9, 15 and 21°C. Furthermore, routine metabolic rates (RMR) were estimated in the control group under acutely rising temperature (3°C/d) and normocapnic and hypercapnic conditions, respectively.
Results indicate that *N. angustata* has a limited thermal tolerance with whole animal critical temperatures at or below 19°C, which is mirrored in very low capacity increases with rising temperature at the mitochondrial level. Acute hypercapnia led to slightly, but not significantly increased RMR, and to higher critical temperatures. Chronic hypercapnia acclimation induced a compensatory up-regulation of mitochondrial capacities, presumably by bicarbonate-mediated intracellular signalling pathways, which was also mirrored in shifts within the metabolome. These enhanced mitochondrial capacities not only result in increased hypercapnia tolerance, but also bring about a higher thermal tolerance at the mitochondrial and whole animal level, which has not been observed in its Antarctic congeners.

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Poster session – Thursday 4th July 2013

A8.29

Hypoxia tolerance and resting metabolism are conserved across genetically distinct sub-populations of an iconic, tropical Australian teleost (*Lates calcarifer*)

Jodie L Rummer (James Cook University, Australia), Geoffrey M Collins (James Cook University, Australia), Timothy D Clark (Australian Institute of Marine Sciences, Australia) and Alexander G Carton (James Cook University, Australia)

Extreme temperatures and altered freshwater flow regimes associated with climate change are predicted to cause large-scale fish mortalities in Northern Australia by increasing the frequency and severity of hypoxic episodes. Currently, there is a lack of understanding regarding the capacity of Australian fish species to respond to such hypoxic events. Furthermore, it is unclear if intraspecific phenotypic variability in performance traits such as aerobic metabolism and hypoxia tolerance are conserved across genetically distinct sub-populations. Here, we used the iconic barramundi (*Lates calcarifer*) as a model species to examine resting oxygen consumption rates (MO_2) and tolerance to acute hypoxia in five different sub-populations spanning 12° of latitude. Fish were obtained from commercial hatcheries at Gladstone, Townsville, Broome, Karumba and Darwin. Fish were maintained at two temperatures (26°C or 36°C), representing the seasonal thermal range across Australia for this species. All populations exhibited a common and clear trend in response to decreasing oxygen tension, with fish maintaining a constant MO_2 between 100% and 30% saturation, below which MO_2 exhibited a steep decline. Mean critical oxygen tension ($[O_2]_{crit}$) across all populations was lower at 26°C (15.44±3.20% saturation) than at 36°C (21.07±3.92% saturation). Resting MO2 was lower at 26°C (mean = 1.46±0.26 mg O_2 kg_{-1} min^{-1}) than at 36°C (mean = 3.10±0.43 mg O_2 kg^{-1} min^{-1}). Overall, we found that both hypoxia tolerance and aerobic resting metabolism are conserved across the distribution of barramundi in Australia.

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Poster session – Thursday 4th July 2013

A8.30

Fasted juvenile garden dormice use short torpor bouts to grow and fatten during pre-hibernation: Consequence on winter hibernation

Sylvain Giroud (Research Institute of Wildlife Ecology, Austria), Ines Hofer (Research Institute of Wildlife Ecology, Austria), Christopher Turbill (University of Western Sydney, Australia) and Thomas Ruf (Research Institute of Wildlife Ecology, Austria)

Prior to hibernation, juvenile hibernators have to sustain both somatic growth and fattening to reach a sufficient body mass to survive the following winter season. This high demand for energy is especially challenging for juveniles born late in the season, since they might already experience reduced food availability and decreasing temperatures. This study investigated: (1) how the use of torpor enables them to compensate the energy deficit, to maintain rates of body mass gain and levels of pre-hibernation fattening similar to well-fed juveniles; and (2) consequences on hibernating patterns and body composition changes over-winter. We quantified torpor use, measured food intake, body mass and pre-hibernation fat level in late born juvenile dormice exposed to intermittent fasting (n=9) or fed *ad libitum* (AL, n=9) under natural photoperiod and ambient temperature for 7 weeks prior to hibernation. We further followed the hibernating patterns and determined their body composition after 3 months of hibernation. We found that fasted juveniles during pre-hibernation extensively used torpor, with lengthening duration as the frequency increased. Fasted juveniles gained body mass at a same rate and reached a fat level prior to hibernation similar to AL juveniles. During the subsequent winter, hibernating patterns and over-wintering body losses did not differ between the two groups. However, both groups of juveniles reduced in the same extent their fat mass and fat-free mass over winter hibernation. Whether a high fat level prior to hibernation is required for juveniles to survive winter hibernation and spare fat-free mass remains to be determined.

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Poster session – Thursday 4th July 2013

A8.31

Biochemical and anatomical responses related to the *in vitro* survival of the tropical bromeliad *Nidularium minutum* to low temperatures

Marcia R Braga (Institute of Botany, Brazil), Camila P Carvalho (Institute of Botany, Brazil), Adriana H Hayashi (Institute of Botany, Brazil) and Catarina C Nievola (Institute of Botany, Brazil)

Nidularium minutum is a tropical bromeliad that grows in natural environment with temperatures from 2 to 30°C. In the present work we cultivated this species *in vitro* at 5, 10, 15, and 25°C for 3 and 6 months aiming at assessing its biochemical and morphological responses that allow its survival under low temperatures. No survival was observed in plants cultured constantly at 5°C and the lowest biometric parameters were found for those grown at 10°C. A thick aquiferous parenchyma, accumulation of reducing sugars, and increased pectin content in the cell walls were observed in plants grown at 10 and 15°C when compared to those from 25°C. These responses indicate the presence of mechanisms related to the maintenance of the water status. In plants cultured at 10°C, leaf bleaching correlated with low chlorophyll content and lower survival after six months when compared to those grown at 15°C. Maintenance at 15°C resulted to be the best *in vitro* culture condition for slow growth and plant acclimatization. This probably correlated with the immediate availability of carbon to restore growth during acclimatization and also with higher root initiation under this condition. This study brings information about the responses related to cold adaptation in *N. minutum* cultured *in vitro* that can be also implicated to its survival under natural conditions. Additionally, it suggests the best temperature to form a minimal growth collection to be used in restocking and conservation programmes for endangered tropical bromeliads (FAPESP).

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Poster session – Thursday 4th July 2013

A8.32

Heat shock response of the lake whitefish (*Coregonus clupeaformis*): A novel model for the adaptation to changing environments and industrial thermal effluents

Richard G Manzon (University of Regina, Canada), Daniel Stefanovic (University of Regina, Canada), Katehrine J Sessions (University of Regina, Canada) and Lori A Manzon (University of Regina, Canada)

Lake whitefish (*Coregonus clupeaformis*) are broadly distributed throughout Canada and the Northern United States and are a species of important socioeconomic value. Whitefish normally occupy deep, cold waters (11–13°C) and spawn in the late autumn/early winter in the near-shore habitat where embryos develop at 1–4°C. As a cold water-adapted species, whitefish are sensitive to increased lake water temperatures and embryos may be susceptible to industrial thermal effluents deposited on the spawning grounds. We hypothesize that exposure to small, transient thermal stress (+1–3°C) during embryogenesis will have protective or adaptive effects in juveniles via shifts in the amounts of constitutive and/or inducible forms of heat shock proteins (HSPs). These shifts may be adaptive in nature and offer protective benefits against more intense stressors. Preliminary work suggests that the levels of constitutive HSPs (HSC70 and HSP90β) are elevated in embryos exposed to regular (weekly, bi-weekly) low-level heat stress. The long term impacts of this embryonic thermal stress in juveniles are being assessed. In addition to having numerous deep lakes, Saskatchewan, Canada is home to many shallow lakes that do not stratify and can reach temperatures of 22–25°C in the summer months. These shallow lakes offer no cool water refuge, yet they sustain stable lake whitefish populations. By studying whitefish from these two distinct lake types, we are identifying key physiological and genetic adaptations in response to thermal history. Data from these wild populations will offer insight into the potential biological consequences of changing environments in this thermally-sensitive aquatic species.

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Poster session – Thursday 4th July 2013

A9: AQUATIC LIFE IN A WARMER AND HIGHER CO_2 WORLD

Organized by: Rod Wilson (University of Exeter) and Fredrik Jutfelt (University of Gothenburg)

A9.1
Ocean acidification and marine fishes

Philip L Munday (James Cook University, Australia)

Ocean acidification, caused by the uptake of additional CO_2 from the atmosphere, is a serious threat to marine ecosystems. In general, marine fishes are thought to be relatively tolerant to rising CO_2 levels and ocean acidification because they have well developed physiological mechanisms for acid–base regulation. However, it was recently discovered that near-future CO_2 levels interfere with a range of sensory functions and behaviours of marine fishes. Behavioural changes include increased activity and boldness, loss of lateralization in movement, altered auditory preferences, and impaired olfactory function.
In this talk I describe the consequences of these sensory and behavioural changes to predator–prey interactions, homing ability and habitat selection in reef fishes, all of which influence the replenishment and sustainability of fish populations. I then describe the underlying mechanism responsible for these diverse sensory and behavioural effects, and how it relates to the exceptional acid–base regulatory abilities of marine fish. New experiments show that exposure to elevated CO_2 interferes with brain neurotransmitter function in marine fish, and possibly other organisms, a previously unrecognized threat of ocean acidification.
I conclude by examining the necessity to test the potential for acclimation and adaptation in ocean acidification research. Short-term experiments have identified many of the ecological impacts of ocean acidification, but an evolutionary perspective is now required to predict the long-term consequences for marine ecosystems.

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10:20 Thursday 4th July 2013

A9.2
An integrated view of climate sensitivity in marine organisms: Linking molecular- to ecosystem-level changes

Hans-Otto Pörtner (Alfred-Wegener-Institute, Germany)

Climate change effects on marine ecosystems predominantly involve effects of temperature, hypoxia and CO_2. All life forms respond to these drivers, potentially following common, insufficiently understood principles. In animals the concept of oxygen and capacity dependent thermal tolerance (OCLTT) appears as a suitable integrator of various effects, across levels of biological organization.
Recent studies confirm OCLTT involvement in changing species abundance, biogeographical ranges, phenology and species predominance. Performance capacity set by aerobic scope and energy budget, building on baseline energy turnover, link fitness (within thermal window) and functioning at ecosystem level. In variable environments like the intertidal zone, animals also exploit their capacity for passive tolerance. The complexity of molecular adjustments involved in long-term adjustments to climate requires further analysis. While presently the temperature signal appears predominant in the field, observations may well include emerging effects of other stressors, acting synergistically by narrowing the aerobic OCLTT window. OCLTT may link apparently disjunct effects of ocean warming, acidification and hypoxia.
In brief, warming induced CO_2 accumulation in body fluids links to the effects of ocean acidification mediated by the weak acid distribution of CO_2. Temperature-induced hypoxemia links to the hypoxia sensitivity of thermal tolerance.
Future work will need to develop proxies for the temperature-dependent effects of climate related stressors and to also identify the principles operative in organisms other than animals and their underlying mechanisms. Mechanism-based modelling efforts are then needed to develop reliable, organism to ecosystem projections of future change.

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11:00 Thursday 4th July 2013

A9.3
Can coastal calcifiers adapt to a future, more acidic ocean?

Frank Melzner (GEOMAR Helmholtz Centre for Ocean Research, Germany)

Seasonally hypoxic coastal ecosystems are already acidified today: respiratory processes go along with the production of carbon dioxide. Hypoxic systems frequently experience seawater pCO_2 values of >1,000 µatm, hence greater values that we expect during the next century for the global surface ocean. Future changes in pCO_2 due to equilibration of the ocean with anthropogenic CO_2 ('ocean acidification') are non-linear in such habitats, leading to frequent occurrence of pCO_2 values >2,000 µatm, particularly in brackish habitats, such as the Baltic Sea (Melzner et al., 2013 *Mar Biol* online early).
However, communities in seasonally acidified coastal systems are often dominated by calcifying species, indicating that high fluctuations in pCO_2 can be compensated to some degree. Here, I will discuss the impacts of high CO_2 on acid–base regulation and calcification abilities in larval and adult stages of molluscs (*Mytilus* spp.) and echinoderms (*Strongylocentrotus droebachiensis*) that inhabit such coastal habitats. Further, I will discuss how changes in acid–bases status could impact energy budgets and species fitness. I also will compare laboratory and field results and discuss the need for more complex experimental approaches to estimate vulnerabilities of species and communities to anticipated ocean change.

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11:30 Thursday 4th July 2013

A9.4

Behavioural and physiological effects of CO_2 exposure in temperate fish

Fredrik Jutfelt (University of Gothenburg, Sweden), Leon Green (University of Gothenburg, Sweden), Karine Bresolin De Souza (University of Gothenburg, Sweden), Maria Hedgärde (University of Gothenburg, Sweden), Joachim Sturve (University of Gothenburg, Sweden), Trond Amundsen (NTNU, Norway), Sam Dupont (University of Gothenburg, Sweden) and Elisabet Forsgren (NINA, Norway)

Fish are generally considered tolerant against the modest CO_2-challenge that future ocean acidification may pose, and very few physiological effects have been demonstrated after exposure to ocean acidification-relevant CO_2 levels. However, several species of coral reef fish show altered behaviours when exposed to CO_2.
Here we show that temperate fish can also develop behavioural abnormalities from long-term CO_2 exposure (1,000 µatm). Two-spotted goby larvae increased phototaxis and activity, three-spined sticklebacks decreased curiosity and lateralization, small-spotted catsharks changed the swimming pattern from a complex behaviour to continuous swimming. In contrast to these behavioural shifts, juvenile Atlantic cod appeared CO_2-tolerant.
After one month of exposure the cod behaved normally in several behavioural tests, yet when given a choice, even the CO_2-acclimated cod strongly preferred control water, demonstrating that habituation to the irritant effect of CO_2 may be very slow.
In addition to behaviour, the effects of CO_2 on physiological performances such as embryonic malformations, pH-regulation and aerobic scope will be discussed.

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12:00 Thursday 4th July 2013

A9.5

Ocean acidification reverses competition for space as habitats degrade

Mark I McCormick (James Cook University, Australia) and Philip L Munday (James Cook University, Australia)

How fish communities are affected by CO_2-induced climate change will be depend on the ability of species to tolerate or adapt to the new conditions, and how the altered characteristics of species influences the outcomes of key processes, such as competition and predation. Research on fish demonstrates that elevated CO_2 causes major changes in behaviour and performance for individuals in isolation. However, it is unknown how these conditions are likely to influence the biological interactions that form the foundation of community dynamics. These effects will not occur in isolation, but rather within habitats that are changing in resource quality and distribution.
The present study examines how levels of CO_2 predicted to occur in the next 70–100 years (950 µatm) may affect the interactions between two damselfish species known to compete for space at settlement in healthy live coral, and coral degraded through thermal bleaching. Species differed in their tolerance to the effects of elevated CO_2 on their behaviour, with the species that is competitively dominant under present day conditions being most affected. Short term (three-day) field experiments showed that elevated CO_2 reversed the competitive outcome between the two species with mortal consequences, and this reversal was accentuated in degraded habitats.
This study emphasises the importance of understanding the tolerance levels of species to elevated CO_2, and its behavioural outcomes, before we can predict the likely composition of future communities.

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12:20 Thursday 4th July 2013

A9.6

Potential cellular mechanisms involved in sensing ocean acidification and triggering physiological responses

Martin Tresguerres (Scripps Institution of Oceanography, UCSD, United States)

Physiological responses to ocean acidification (OA) are highly variable and species-specific. For example, OA has been reported to induce negative, neutral, or even positive effects on biological calcification, as well as on metabolism, reproduction and behaviour in diverse organisms. Moreover, other studies have found significant interactions between OA, temperature, and feeding. These observations suggest that at least some physiological responses to OA are determined by the acid–base and metabolic statuses, and that at least some organisms have the capacity to sense OA and regulate their physiology accordingly. Additionally, the compensatory response to hypercapnic acidosis of many animals involves accumulating bicarbonate in physiological fluids. It is thus possible that some of the physiological responses to OA are in reality triggered by compensatory metabolic alkalosis.
The enzyme soluble adenylyl cyclase (sAC) has been implicated in sensing metabolic and environmental CO_2, pH and bicarbonate ion in diverse species ranging from cyanobacteria to mammals. sAC produces the messenger molecule cAMP, which can modulate multiple aspects of biology via post-translational modifications on target proteins. Using genomic, transcriptomic, proteomic and/or enzymatic methods, we have detected sAC in four species of corals, in oyster mantle, gill and haemocytes, and in multiple fish species and tissues.
We are currently investigating the roles of sAC and the cAMP signal transduction pathway in regulating physiological responses to OA, including pH regulation, calcification, and gene expression.

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12:40 Thursday 4th July 2013

A9.7

Intraspecific variation in thermal and hypoxia tolerance in fish: Implications for responses to a changing climate

Patricia M Schulte (University of British Columbia, Canada)

Although evolutionary change in populations has traditionally been considered to be a slow process, occurring over the course of millennia, recent studies suggest that evolution can occur much more rapidly, which opens the possibility of adaptive evolution in response to anthropogenic climate change. The existence of standing genetic variation for climate-relevant traits is likely to be important in allowing populations to respond to rapid climate change through adaptive evolution, but the extent of such variation in natural populations remains unclear. For example, in aquatic environments, climate change is resulting in increases in both temperature and prevalence of hypoxia.
In order to adapt to these combined stressors, populations would have to harbour extensive intraspecific variation in tolerance to both of these stressors, and this variation would have to co-vary positively. Recent work in my laboratory has demonstrated that there is extensive genetically-based intraspecific variation in thermal and hypoxia tolerance in several species of temperate zone fishes, and that these traits co-vary positively. This type of variation should promote the ability of fish to respond to these co-varying climate stressors through adaptive evolution.

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13:55 Thursday 4th July 2013

A9.8
Coral reef life getting even warmer

Göran E Nilsson (University of Oslo, Norway)

Average sea-surface temperatures are projected to rise considerably in a near future due to increasing concentrations of atmospheric green house gases, particularly CO_2. Many coral reef fishes are already living close to their thermal optimum, and for some of them, even relatively moderate increases in temperature (2–4°C) lead to significant reductions in aerobic scope (i.e. the difference between resting and maximum rates of oxygen uptake) and hypoxia tolerance. Reduced aerobic capacity could affect population sustainability since less energy can be devoted to feeding and reproduction.

Many reef fishes seek shelter from predators at night in the coral matrix, where nocturnal hypoxia is common. Losing hypoxia tolerance may therefore reduce their ability to avoid predation. Finally, the ability to cope with severe hypoxia by utilizing anaerobic pathways is impaired at higher temperatures, suggesting that reduced anaerobic scope, in addition to reduced aerobic scope, will be a threat to some species in a warmer future.

It is uncertain if species that have lived in a stable climate for a long time will possess a gene pool that is diverse enough to allow natural selection to adapt them to a substantial increase in temperature over just a few decades. However, there may still be some hope. Although coral reef fish seem to have limited capacity to acclimate to elevated temperatures as adults, recent research shows that developmental and transgenerational plasticity occur, which might enable some species to adjust to rising ocean temperatures.

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14:25 Thursday 4th July 2013

A9.9
Constructing a trait-based index of sensitivity to predict the response macrobenthic community structure and function to elevated CO_2

Steve Widdicombe (Plymouth Marine Laboratory, United Kingdom) and Martin Solan (University of Southampton, United Kingdom)

In response to the pressing environmental challenges posed by rising levels of CO_2 in the marine environment (e.g. ocean acidification) and the potential impacts of some geoengineering climate change reduction technologies (e.g. geological and ocean carbon sequestration), the past decade has seen the publication of many studies exploring the potential impacts of elevated CO_2 on the health and function of marine organisms.

Given this growing body of data and understanding, are we now able to predict the relative sensitivity of any particular species to CO_2 based solely on knowledge of its physiology, ecology and behaviour? This was the question posed at a recent workshop funded by the Natural Environment Research Council's UK Ocean Acidification Programme.

A group of cross-discipline researchers was brought together to use existing data and expert opinion to construct a trait-based index of CO_2 sensitivity. The index was then tested against existing community level data from mesocosm experiments and observational studies at sites with naturally high levels of CO_2. Finally, the sensitivity index was used to construct simple models of species extinctions and predict the likely impact of elevated CO_2 on biodiversity and community function.

This presentation will detail the methodology employed to construct and test the trait-based index of CO_2 sensitivity and will explore the potential for this approach to predict ecosystem level consequences.

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15:35 Thursday 4th July 2013

A9.10
Measuring temperature tolerance at the most appropriate scale: The hierarchy of response loss

Simon A Morley (British Antarctic Survey, United Kingdom), Amanda E Bates (University of Tasmania, Australia) and Lloyd S Peck (British Antarctic Survey, United Kingdom)

The global redistribution of species has focussed interest on the mechanisms underlying where species can and cannot live. Large-scale geographic studies of physiological tolerance have led to the development of several macrophysiological principles, including the metabolic theory of ecology and oxygen and capacity limitation. While patterns in physiological traits are emergent at a global scale, fine-scale microhabitat and temporal variability are also important. Thus the mechanisms conferring heat tolerance are shaped by evolution and ecology over multiple scales. Within organisms, there is an expected hierarchy of upper temperature limits, which are predicted to reduce with increasing complexity from biochemical pathways through to whole animals. Here we test for latitudinal patterns in this hierarchy of response and predict that: heat tolerance will reduce with increasing biological complexity; broad patterns in the hierarchy of heat tolerance will reflect climate; and animals from habitats with greater variability will have a wider range of response hierarchy. We test if different upper temperature limits (lethal, critical, activity) and optimum temperatures relate similarly with latitude. We summarize recent work on upper temperature limits in limpet species, including new measures of activity and feeding rate. Then, by combining literature data from studies that measured incipient upper lethal and upper critical temperatures using consistent methodologies we provide strong evidence that the compression of temperature limits in tropical species is a general response. Our study indicates that different mechanisms appear to underlie different temperature limits in the ocean, as shaped by mean temperature and variability.

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16:05 Thursday 4th July 2013

A9.11
Fish physiology at the nuclear power plant: What can we learn about the long-term responses to climate change?

Erik Sandblom (University of Gothenburg, Sweden), Andreas Ekström (University of Gothenburg, Sweden), Aho Teija (Swedish University of Agricultural Sciences, Sweden), Fredrik Sundström (Uppsala University, Sweden) and Fredrik Jutfelt (University of Gothenburg, Sweden)

Global climate change is predicted to continue with increasing average temperatures and more frequent summer heat spells. This will have a profound impact on fish where the body temperature and metabolism is determined by the ambient temperature. Current hypotheses suggest that oxygen transport limitations may constrain ectothermic animals in a warmer future. Yet, knowledge about the long-term adjustments to the cardiorespiratory system in fish via acclimation and genetic adaptation to chronic warming is limited. Results from a recently launched project on cardiovascular and respiratory function in Eurasian perch (*Perca fluviatilis*) that live and reproduce in the chronically heated 'Biotest enclosure' in the Baltic Sea off the nuclear power plant in Forsmark in Sweden will be presented and discussed. Cooling water from the nuclear reactors has been directed to the 90 hectare enclosure since 1980 keeping water temperatures 5–10°C above ambient for over 30 years, but at otherwise similar abiotic conditions as the surrounding archipelago. Thus, this experimental facility presents a unique opportunity to study the physiological responses to long-term warming in a wild fish population. Our results reveal that fish from the heated enclosure have smaller hearts and are considerably more tolerant to acute temperature increase and better able to maintain cardiac function during acute thermal challenges relative to fish from outside the enclosure. In addition, they have a depressed metabolic rate and a lower resting heart rate due to a higher cholinergic tone and a reduced intrinsic cardiac pacemaker.

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16:25 Thursday 4th July 2013

A9.12
Cognitive impairment in coral reef fishes living in shallow water volcanic CO_2 seeps

Danielle L Dixson (Georgia Institute of Technology, United States), Jodie L Rummer (ARC Centre of Excellence for Coral Reef Studies, Australia), Alistair J Cheal (Australian Institute of Marine Science, Australia), Katharina E Fabricius (Australian Institute of Marine Science, Australia) and Philip L Munday (James Cook University, Australia)

There is growing concern that ocean acidification will severely impact marine ecosystems. Calcifying organisms have been shown to suffer the expected negative effects of acidification but non-calcifiers may be equally threatened. Lab experiments have demonstrated that fish behaviour alters as CO_2 levels increase; individuals display impaired sensory systems and behavioural lateralization along with changes in activity, boldness and habitat use. It is difficult to extrapolate findings from short-term laboratory studies to naturally occurring communities and to determine the effects of long-term acclimation to elevated levels of CO_2. Shallow coral reefs in parts of Papua New Guinea experience CO_2 levels similar to those predicted under end-of-century models due to naturally occurring volcanic seeps. We investigated the chemosensory system and behaviour (activity level, habitat use, boldness and brain lateralization) of five fish species collected from seep and control locations. Differences between treatments were marked. Unlike control fishes, those collected in high CO_2 sites were unable to recognize and respond appropriately to predator and habitat cues, displayed non-lateralized turning patterns and doubled the time spent away from refuge sites. For example, control individuals completely avoided predator cues, while fish collected from CO_2 sites spent >90% time in the predator cue, similar to results recorded in short-term laboratory experiments. Overall, fish from high CO_2 sites showed an increase in activity and boldness. We conclude that long-term acclimation does not reverse behavioural impairment and results gained from short-term laboratory behaviour studies may reflect the sensitivity of natural fish communities to elevated CO_2.

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16:45 Thursday 4th July 2013

A9.13
Regulation of intracellular pH in cnidarians: response to acidosis in *Anemonia viridis*

Julien Laurent (Centre Scientifique de Monaco [CSM], Monaco), Alexander Venn (CSM and Laboratoire Européen Associé 647 “Biosensib” CSM-CNRS, Monaco), Éric Tambutté (CSM and Biosensib CSM-CNRS, Monaco), Philippe Ganot (CSM, Monaco), Denis Allemand (CSM and “Biosensib” CSM-CNRS, Monaco), Sylvie Tambutté (CSM and “Biosensib” CSM–CNRS, Monaco)

The regulation of intracellular pH (pHi) is a fundamental aspect of cell physiology that has received little attention in studies of symbiotic cnidarians. Most cellular activities including enzyme activity and vesicle trafficking, are highly pH-sensitive. Anthozoans must maintain pH homeostasis to counterbalance falls in intracellular pH which can arise due to changes in either intrinsic or extrinsic parameters. Coral cells and sea anemones’ cells face natural daily changes in extracellular pH which can vary from 7.4 at night to 8.9 during the day. Moreover, species of this phylum are threatened by ocean acidification. We investigated pHi regulation in endodermal cells of *Anemonia viridis*, an emerging model for understanding cell biology in the phylum Cnidaria. To gain insights into the process of pHi regulation we used a physiological approach with pH sensitive dyes and confocal live cell imaging .We characterized pHi recovery after intracellular acidosis induced by NH_4 Cl, HCl or CO_2. We investigated the importance of Na^+/H^+ plasma membrane exchangers (NHEs) necessary for recovery after intracellular acidosis. We also measured the buffering power of cells and obtained values between 20.8 and 43.8 mM/pH, comparable with values obtained in other invertebrates. Our findings on pHi regulation constitute an important step toward a better comprehension of acid–base regulatory abilities of anthozoans which is imperative for a better grasp of cnidarian physiological behaviour, especially in the context of ocean acidification.

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Poster session – Thursday 4th July 2013

A9.14
Potential mechanisms for pH sensing and regulation in corals

Katie L Barott (Scripps Institution of Oceanography, United States), Megan Barron (Scripps Institution of Oceanography, United States) and Martin Tresguerres (Scripps Institution of Oceanography, United States)

Maintaining acid/base homeostasis is critical for all forms of life, and is increasingly important for corals facing ocean acidification. While it is known that corals increase extracellular pH at the site of calcification while undergoing daily fluctuations in intracellular pH due to photosynthesis, little is known about how corals sense or regulate these changes. One potential mechanism we investigate here is the enzyme soluble adenylyl cyclase (sAC), which is directly stimulated by bicarbonate to produce cAMP in many organisms. In mammals and sharks, sAC activity modulates proton pumps (V-type H^+-ATPase [VHA]), which compensate for changes in blood pH.
Investigation of the *Acropora digitifera* genome revealed two genes encoding sAC in corals, as well as genes for VHA. In live corals, endogenous cAMP levels were higher during the day compared to the middle of the night, indicating a diel cycle, and bicarbonate-stimulated cAMP production was detected in homogenized coral tissue as well as purified recombinant coral sAC.
These results support the hypothesis that sAC is an important regulator of coral physiology, especially in response to light, acid–base disturbances, and inorganic carbon levels. Finally, VHA was detected in corals and localized to the host membrane enveloping the zooxanthellae.
We propose that VHA plays a role in regulating intracellular acid–base conditions in response to or in promotion of photosynthesis and as a carbon concentrating mechanism. These results are the first step towards understanding the cellular mechanisms of acid–base regulation in corals, and are critical for predicting how corals will respond to ocean acidification.

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Poster session – Thursday 4th July 2013

A9.15
Why fish brains are affected by high CO_2

Floriana Lai (University of Oslo, Norway), Fredrik Jutfelt (University of Gothenburg, Sweden) and Goran E Nillson (University of Oslo, Norway)

The general increase in CO_2 levels in the atmosphere is now starting to affect aquatic organisms and could have consequence for various physiological functions. Several studies have identified drastic effects of near-future CO_2 levels on the behaviour of coral reef fish, effects that can be rapidly reversed by the exposure to gabazine, a GABA-A receptor antagonist. This study aims at clarifying the molecular background to ion-regulatory changes in the brain during high-CO_2 exposure that affect the function of the GABA-A receptor, including the expression of Cl^- transporters presents in neurons.
Regulatory changes of HCO_3^- and Cl^- ions across the neural membrane could render some GABA-A receptors excitatory rather than inhibitory thereby affecting fish behavior. While previous findings were made on coral reef fish, there are no strong reasons to assume that these phenomena would be limited to reef fishes. Studies of altered behaviours and of the expression of GABA-A receptor subunits and Cl^- transporters made on a temperate fish, the three-spined stickleback (*Gasterosteus aculeatus*) will be presented, providing worrying evidence that near-future CO_2 levels can directly affect neurotransmitter system in fish brains with drastic and possibly widespread effects on their survival.

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Poster session – Thursday 4th July 2013

A9.16

The importance of parents: Transgenerational acclimation ameliorates the negative impacts of high CO_2 in a coral reef fish

Gabrielle M Miller (James Cook University, Australia), Sue-Ann Watson (James Cook University, Australia), Cecilia Villacorta (James Cook University, Australia), Rhondda Jones (James Cook University, Australia), Mark I McCormick (James Cook University, Australia) and Philip L Munday (James Cook University, Australia)

Hgh CO_2 and ocean acidification can negatively impact growth, behaviour and survival of marine organisms, yet most ocean acidification studies have not considered the potential for parental effects to alter organismal responses. We used a multi-generational experiment to determine the parental effects as a result of increased CO_2 on the growth, survival, routine metabolic rate (RMR) and otolith development of juvenile cinnamon anemonefish, *Amphiprion melanopus*. Wild-caught breeding pairs were held for a 9-month period, inclusive of the austral summer breeding season, under control (430 µatm), moderate (~600 µatm) and high (~1,000 µatm) CO_2 and allowed to spawn naturally under these conditions. Egg clutches were then either hatched into their parental CO_2 conditions, or some clutches from control parents were reared in high CO_2 from hatching. Control-to-high juveniles were significantly smaller, had lower survival and higher RMR compared to the control group. In contrast, offspring reared at moderate and high CO_2 and whose parents also experienced elevated CO_2 were of comparable size, and had similar survival and RMR to control juveniles. Otoliths from control-to-high juveniles were on average larger and less variable in size than the control group. These differences were not apparent in the groups at parental CO_2 conditions, suggesting that parental effects restore the natural variation in otolith calcification that is absent in the control–high group. Our results show ignoring parental effects and the potential for transgenerational acclimation may lead to an overestimation of negative impacts of ocean acidification in marine fishes.

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Poster session – Thursday 4th July 2013

A9.17

Biomineralization: Protein and mineral response of *Mytilis edulis* to combined ocean acidification and increasing seawater temperatures

Susan C Fitzer (University of Glasgow, United Kingdom), Vernon Pheonix (University of Glasgow, United Kingdom), Maggie Cusack (University of Glasgow, United Kingdom) and Nick Kamenos (University of Glasgow, United Kingdom)

Ocean acidification (OA) poses a problem for calcareous organisms which utilize carbonate to produce their exoskeletons. The combination of increasing temperature and pCO_2 has the potential to synergistically impact calcifying organisms. The common blue mussel *Mytilus edulis* combines the two polymorphs of calcium carbonate ($CaCO_3$), calcite and aragonite into their shells. A pH reduction associated with OA will bring about the dissolution of calcium carbonate minerals, with aragonite being more susceptible than calcite. While declining carbonate concentrations at the sea surface threatens marine biogenic growth, living organisms can control the production of biominerals through proteins expressed for growth. We investigated seasonal long-term ocean acidification and increased seawater temperature impact on the protein and mineral components of *M. edulis*. We assessed changes in carbonic anhydrase (CA) concentration and activity, a key protein in biomineralization, catalysing the hydration of CO_2 required to form $CaCO_3$. Trends indicated a reduction in CA concentration for 1,000 ppm treatments compared to ambient 380 ppm. Conversely, the CA activity was much higher in those mussels grown under 1,000 ppm compared to ambient conditions. Similar trends were observed in extrapallial fluid and mantle tissue CA activity, with increased activity at 550 ppm and reduced activity at 750 ppm compared with ambient 380 ppm activity. Mussel shell growth was lower in mussels grown in 1,000 ppm compared to 380 ppm.

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Poster session – Thursday 4th July 2013

A9.18

Oxygen delivery is not limited at high temperatures in three species of eurythermal crustaceans

Rasmus Ern (Aarhus University, Denmark), Do Thi Thanh Huong (Can Tho University, Vietnam), Nguyen Thanh Phuong (Can Tho University, Vietnam), Tobias Wang (Aarhus University, Denmark) and Mark Bayley (Aarhus University, Denmark)

Rising water temperatures unavoidably reduce water oxygen content while oxygen demand of the organism increases. To investigate whether high temperatures constrain the cardiorespiratory system and thereby determine upper tolerable thermal limits, we studied three species of eurythermal crustaceans (*Astacus astacus*, *Macrobrachium rosenbergii* and *Penaus monodon*) at temperatures at and above their normal rearing temperatures.

From measurements of heart and ventilation rates, resting and maximum oxygen uptake, critical upper temperatures during normoxia and hypoxia, as well as haemolymph lactate concentrations, we show that oxygen transport capacity in these animals is maintained up to temperatures immediately below their critical upper temperatures. Further, the giant freshwater shrimp (*Macrobrachium rosenbergii*) maintains normal growth when challenged by a temperature rise of 6°C. Thus, in these species that are adapted to high environmental temperatures, either during summer months (the temperate *A. astacus*) or throughout the year (the tropical *M. rosenbergii* and *P. monodon*), the cardiorespiratory system has adapted to meet oxygen demand at high temperatures and there is no evidence that their upper thermal limits are dictated by the collapse of adequate oxygen transport. Hence, none of the three species fit with the currently accepted paradigm that the upper thermal boundary for the distribution and ecological performance of aquatic poikilotherms is determined by oxygen delivery, and we propose that other mechanisms such as protein dysfunction must underlie the upper temperature boundary.

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Poster session – Thursday 4th July 2013

A9.19

Compromised development of flatfish (*Solea senegalensis*) larvae under ocean warming and acidification

Marta S Pimentel (Universidade de Lisboa, Portugal), Filipa Faleiro (Universidade de Lisboa, Portugal) and Rui Rosa (Universidade de Lisboa, Portugal)

Early life stages are expected to be the most vulnerable to climate changes, but the knowledge of their capacity to cope under such conditions remains poorly understood. The present study investigates the synergistic effects of ocean warming (18ºC – the average summer temperature at western Portuguese coast – and 22°C – a near-future warming scenario of +4ºC) and acidification (present pH 8.0 and future pH 7.5) on the early development of the flatfish *Solea senegalensis*, namely on hatching success rates, larval survival and growth rates, skeletal abnormalities, otoliths size, metabolism and thermal tolerance limits.

Both hypercapnia and warming elicited significant deleterious effects on the hatching success, larvae survival and growth. Ocean acidification also accelerated larvae otolith growth and induced severe skeletal abnormalities, e.g. cranium anomalies, lordosis, scoliosis and kyphosis. Oxygen consumption rates increased significantly with warming ($p<0.05$) and OA led to larval metabolic suppression. The thermal tolerance limits were significantly affected by temperature, pCO_2 and developmental stage ($p<0.05$). Lower thermal tolerance limits of early larvae seem to be strictly connected to high metabolic demands associated with the planktonic life strategy.

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Poster session – Thursday 4th July 2013

A9.20

Impaired enzymatic activity and increased peroxidative damage of flatfish (*Solea senegalensis*) larvae under ocean warming and acidification

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Here we investigated the effect of ocean warming (18ºC – the average summer temperature at western Portuguese coast – and 22°C – a near-future warming scenario of +4ºC) and acidification (present pH 8.0 and future pH 7.5) on the digestive and antioxidant enzymatic activities, lipid peroxidation and heat shock response (HSP70) of flatfish larvae (*Solea senegalensis*).
The digestive enzyme (pancreatic and intestinal) activities, namely trypsin, amylase, aminopeptidase and alkaline phosphatase were significantly affected by ocean warming and acidification ($p<0.05$). As expected, the enzymatic activity increased with temperature, but decreased with lower pH. Under the future conditions, an increment of malondialdehyde levels was observed ($p<0.05$), indicating that the larvae were subjected to a peroxidative damage during thermal and hypercapnic acclimation. The HSP70 concentration was also highest under the future scenarios, especially under lower pH conditions ($p<0.05$). Moreover, there was a significant increase in catalase (CAT) and glutatione S transferase (GST) activities. Thus, HSP70, CAT and GST may constitute an integrated physiological stress response to these climate-related environmental changes in flatfish larvae.

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Poster session – Thursday 4th July 2013

A9.21

Physiological and behavioural responses to CO_2 exposure in the temperate Small-spotted catshark

Leon Green Ekelin (University of Gothenburg, Sweden) and Fredrik Jutfelt (University of Gothenburg, Sweden)

Due to anthropogenic CO_2 emissions, the pH of the ocean is currently being lowered at a previously unseen rate. Despite efficient pH-regulation, teleost fish can be affected by near-future levels of pCO_2. Acidosis is countered by abnormally high HCO_3^- concentrations. This has been hypothesized to account for the behavioural shifts seen in several studies of tropical teleosts. Few studies have been made on the effect of ocean acidification on temperate fish, and no work has so far been published on the effect on elasmobranchs.
In this study we analyse the effect of long-term exposure to 1,000 µatm CO_2 on the temperate Small-spotted catshark (*Scyliorhinus canicula*). The high CO_2 exposure did not significantly affect survival, growth, haemoglobin concentration, resting metabolic rate or aerobic scope. However, plasma Na^+ and HCO_3^- concentrations were significantly increased. Behavioural trials showed an increase in lateralization, which contrasts to previously published results on teleosts. Sharks exposed to high CO_2 were also found to swim more continuous and with less behavioural variation than untreated sharks. This behavioural change is consistent with the hyperactivity previously found in high CO_2 exposed teleosts.
This study supports the view that the effects of ocean acidification seems to vary among different groups of organisms and adds elasmobranchs to the long list of organisms affected by the increase of atmospheric CO_2.

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Poster session – Thursday 4th July 2013

A9.22

The energetic costs of acid–base regulation in the velvet swimming crab *Necora puber* (L.) during exposure to CO_2 enriched seawater

Lucy M Turner (Plymouth University, UK), Daniel Small (Plymouth University, UK), Nathan Atkinson (Plymouth University, UK), Piero Calosi (Plymouth University, UK), John I Spicer (Plymouth University, UK), Stephen Widdicombe (Plymouth Marine Laboratory, UK) and Samuel PS Rastrick (Plymouth University, UK)

Many studies have found that compared to other taxa, aquatic crustaceans may show greater resilience to environmental change possibly, partly due to their ability to effectively regulate internal ionic concentrations and acid–base balance. However, it is possible that these 'tolerant' species will be adversely affected by elevated pCO_2 due to the energetic trade-offs involved in maintaining their buffering responses. Assessing the functional energetic trade-offs of such organisms is imperative to our understanding of the effects of elevated pCO_2 on marine organisms and ecosystems. The velvet swimming crab *Necora puber* possesses effective acid–base regulatory abilities to short- and medium-term acidification. To attempt to understand the energetic consequences of acid–base regulation in *N. puber*, changes in feeding rates, haemolymph [glucose], hepatopancreas [glycogen] and gill Na^+/K^+-ATPase activity were assessed in animals exposed to elevated pCO_2 conditions (896 and 9,255 µatm) for 30 days (control, 441 µatm). Haemolymph pH significantly decreased, whereas pCO_2 levels and [HCO_3^-] increased in animals exposed to elevated pCO_2 compared to the control group. Na^+/K^+-ATPase activity increased in crabs exposed to high pCO_2, which correlated with an increase in haemolymph [glucose] and decrease in hepatopancreas [glycogen] thus demonstrating the high energetic costs of buffering against elevated pCO_2 even in a 'tolerant' species. Long term, this could prevent investment in maintenance, reproduction and growth in 'tolerant' species, which has far reaching implications for the marine ecosystem.

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Poster session – Thursday 4th July 2013

A9.23

The use of biosensors to monitor physiological parameters in response to environmental and production stress in cultured abalone

Andrea J Morash (University of Tasmania, Australia), Sarah J Andrewartha (CSIRO, Australia), Nicholas G Elliott (CSIRO, Australia) and Peter B Frappell (University of Tasmania, Australia)

Suboptimal animal health is often associated with culturing conditions, and this is predicted to become more prevalent and unpredictable with changing climate. Typically, health is monitored through growth rate and survival which is usually done retrospectively using invasive sampling techniques that do not give any indication of the animal's physiology. The development of *in-situ* biosensors has enabled us to collect real-time data from intensively reared aquaculture species, which has helped fill the gaps that exist in our knowledge of their physiology. In particular, the effects and interactions of environmental (elevated water temperature and decreased oxygen) and production stressors (diet, handling, and air and light exposure) on physiology have been relatively unexplored. Our objective is to determine the physiological coping ranges and responses of temperate abalone to these environmental and production interactions/stressors. We used novel biosensors to measure real-time physiological parameters (heart rate, body temperature and movement) in hybrid greenlip (*H. laevigata*)/blacklip (*H. rubra*) abalone immersed and emersed at 7°C, 15°C and 25°C, in conjunction with metabolic rate and haemolymph biochemical parameters. These experiments have led to the development of preliminary algorithms for the association of changes in physiological parameters (heart rate/metabolic rate) with environmental and production stressors which will guide optimal physiological growth conditions.

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Poster session – Thursday 4th July 2013

A9.24

CO_2 and temperature influences on the physiological responses of Pacific oyster spat to emersion

Sarah J Andrewartha (CSIRO, Australia), Andrea J Morash (University of Tasmania, Australia), Peter B Frappell (University of Tasmania, Australia) and Nicholas G Elliott (CSIRO, Australia)

The aquaculture industry is growing rapidly in both developed and developing regions. Molluscs, such as oysters are farmed both as vital food sources and highly valuable commercial commodities. With industrial growth comes concerns about sustainability (social, biological, economic and environmental) and maximising productivity under a variety of environmental conditions.
It is not only the environmental conditions of today that we need to understand, but also the environmental conditions of the near and coming future. How marine calcifiers (including oysters) respond physiologically to increased temperatures and ocean acidification resulting from the predicted increase in atmospheric CO_2 is a question relevant to small and large aquaculture industries across the globe.
Young oysters in particular are at a vulnerable stage of their life cycle. While an increase in water temperature may be beneficial, increased pCO_2 is detrimental to oyster larvae size, survivorship and metamorphosis (e.g. Talmage and Gobler, 2009). In addition, newly-settled oysters may become emersed from the water (in the field) where they could be exposed to extreme temperatures.
This research examines the effect of temperature and increased pCO_2 on oyster spat metabolic rate and heart rate in and out of water.

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Poster session – Thursday 4th July 2013

A9.24

Physiological acclimation to low level hypercapnia in fish: Associated consequences and possible trade-offs

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Although teleosts are known to be strong acid–base regulators, CO_2 exposure as low as 1,000 matm induces an acidosis in adult toadfish (*Opansus beta*), leading to metabolic compensation by retention of blood HCO_3^- to defend blood pH. Since plasma HCO_3^- and PCO_2 play a role in intestinal HCO_3^- secretion rates, it is important to understand how changes in blood chemistry during ocean acidification could impact intestinal contributions to osmoregulation and acid–base balance.
Toadfish exposed to 1,900 matm CO_2 over 72 hours exhibit higher rectal base excretion rates, higher rectal fluid HCO_3^- (mM), lower Cl^- (mM) compared to 380 (control) exposures, suggesting increased apical anion exchange. These observations confirm that increased base loss occurs in the intestine during exposure to elevated CO_2, a loss that appears counterproductive during whole-body compensation for acidosis.
In order to determine if toadfish dynamically regulate transporters and enzymes associated with HCO_3^- secretion in order to reduce base loss, isolated intestinal tissue from toadfish exposed to control or 1,900 matm CO_2 for two weeks were examined using an Ussing/pH stat system. Contrary to expectations, tissue from CO_2-exposed toadfish exhibited significantly higher base secretion rates than controls, suggesting that these fish do not have the capacity to reduce base loss during prolonged CO_2 exposure. The elevation of HCO_3^- secretion suggests chronic hypercapnia incurs an increased intestinal metabolic cost that could reallocate energy from other life processes, a hypothesis that will be examined by measuring oxygen consumption rates of isolated intestinal tissue in toadfish following chronic CO_2 exposure.

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Poster session – Thursday 4th July 2013

A9.26

pH-sensor molecules in the reef-building coral *Acropora millepora*

Anthony Bertucci (James Cook University, Australia), Aurelie Moya (James Cook University, Australia), Sylvain Foret (Australian National University, Austria) and David J Miller (James Cook University, Australia)

Reef-building corals are among the most prominent marine calcifying organisms. They create ecosystems known for their extraordinary productivity, biodiversity and economic value. A decline in coral cover has been observed on a global scale, favoured by ocean acidification that occurs as a consequence of anthropogenic emissions of CO_2. Recent results have shown that this external acidification directly impacts coral physiology, but also suggest regulation mechanisms allowing the animal to mitigate the effects of this decrease in external pH.
Acid-sensing ion channels (ASICs) are members of the large and multifunctional DEG/ENaC family. They form a family of voltage-independent cation channels that predominantly conduct Na^+ ions. ASICs are activated by extracellular acidification within the physiological range, and are then thought to form effective proton sensors besides their structure and functions are still partially understood. These proteins were primarily restricted to mammal nervous system, but they have recently been identified in a larger set of species, cells and tissues.
By using the whole transcriptome of the coral *A. millepora*, we identified eight members of the DEG/ENaC family among which five show high similarities with ASICs. These proteins may represent a key component of coral sensitivity and physiological response to external pH changes.

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Poster session – Thursday 4th July 2013

A9.27

Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures

Tommy Norin (Aarhus University, Denmark), Hans Malte (Aarhus University, Denmark) and Timothy D Clark (Australian Institute of Marine Science, Australia)

Climate warming is predicted to impact fish populations through impairment of oxygen transport systems when temperatures exceed those which are optimal for aerobic scope (AS). This concept of oxygen- and capacity-limited thermal tolerance (OCLTT) is rapidly gaining popularity within climate change research and has been applied to several fish species. Here, we evaluated the applicability of the OCLTT concept for juvenile barramundi (*Lates calcarifer*) by:

- measuring standard and maximum metabolic rates (SMR and MMR, respectively) and AS of 29°C-acclimated fish acutely exposed to temperatures ranging from 23 to 38°C;
- allowing the fish to behaviourally select a preferred temperature between 29 and 38°C; and
- quantifying alterations to AS after five weeks of acclimation to 29 and 38°C.

A continual increase in MMR with temperature exceeded that of SMR in acutely exposed fish such that AS was highest at 38°C, a temperature approaching the upper lethal limit (40–41°C). Despite 38°C eliciting maximum AS, when given the opportunity the fish maintained a median temperature of 31.7±0.5°C and spent only 10±3% of their time at temperatures >36°C. Measurements at 38°C following acclimation caused a reduction in AS to the same level as 29°C-acclimated fish measured at 29°C, suggesting that AS may be dynamically modulated independent of temperature to accommodate the requirements of daily life. Together, these results reveal limited power of the OCLTT hypothesis in predicting optimal temperatures and effects of climate warming on juvenile barramundi, and question the proposed universality of the OCLTT hypothesis for fishes.

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Poster session – Thursday 4th July 2013

A9.28

Effects of near-future ocean acidification on the larvae of commercially-important finfish

Edward C Pope (Swansea University, UK), Robert P Ellis (University of Exeter, UK), Maria Scolamacchia (Swansea University, UK), Jacob WS Scolding (Swansea University, UK), Alexander Keay (Swansea University, UK), Purazen Chingombe (Swansea University, UK), Robin J Shields (Swansea University, UK), Ceri Lewis (University of Exeter, UK), Rod W Wilson (University of Exeter, UK) and Kevin J Flynn (Swansea University, UK)

There is an acknowledged bias in research on the effects of ocean acidification (OA) towards studies investigating calcifying invertebrates. While the potential effects on these organisms are undoubtedly important, there are relatively few corresponding studies for other taxa. There remains a dearth of information for species of commercial relevance. To address this, we studied the Atlantic herring, *Clupea harengus* (a member of Clupeiformes, the most important fish order for worldwide fisheries in terms of biomass) and European seabass, *Dicentrarchus labrax* (an important fishery and aquaculture species). We exposed the eggs and planktonic larvae of these finfish to combinations of increased temperature and simulated increased atmospheric pCO_2 to investigate potential OA effects on growth and survival. The upper pCO_2 (750 ppm) was selected to comply with the IPCC A2-SRES emission trajectory which predicts atmospheric CO_2 concentrations of between 730 and 1,020 ppm by 2,100, with a projected corresponding decline in oceanic pH from 8.2 to 7.8. To consider potential energetic effects, basal and active metabolic rates were determined in metamorphosed juveniles using closed-system respirometry. In addition, as recent studies have demonstrated aberrant behaviour in juvenile fish under near-future OA conditions, potential behavioural effects were investigated using high-definition video cameras and tracking algorithms. This presentation will discuss the results of these experimental approaches, as well as the importance of selecting appropriate species for ocean acidification research and the providence of the animals used.

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Poster session – Thursday 4th July 2013

A9.29

CO_2-induced ocean acidification increases anxiety in rockfish

Trevor J Hamilton (MacEwan University, Canada), Martin Tresguerres (Scripps Institution of Oceanography, United States) and Adam Holcombe (MacEwan University, Canada)

Atmospheric CO_2 levels have been steadily increasing since the industrial revolution. CO_2 dissolves in water forming hydrogen ions and resulting in ocean acidification (OA). As the rate of anthropogenic OA is faster than any acidification event from the geological past, there are serious concerns on how OA will affect marine life. Fish buffer hypercapnic acidosis by accumulating bicarbonate in their blood, which results in an equimolar reduction in chloride. Previous research has shown that CO_2-induced OA alters fish olfaction by switching the reversal potential of neuronal chloride channels called GABAA receptors. Since GABAA receptors play a major role in anxiety, we examined the potential effect of CO_2-induced OA on anxiety in rockfish using the validated light-dark test (scototaxic assay) that is commonly used in zebrafish models. We exposed rockfish to water with elevated CO_2 (~1,125 + 100 ppm, pH 7.75) for 7 days, and then tested for anxiety on day 8. Results indicate that OA caused rockfish to spend more time in the dark zone, an indication of increased anxiety, relative to control fish (~483 + 40 ppm CO_2, pH 8.1). The application of the GABAA receptor antagonist gabazine caused a significant increase in anxiety in the control fish, but not in the already anxious CO_2 group. After the scototaxic assay we placed the CO_2 group back into normal ocean water and retested for anxiety 7 and 12 days later. Fish remained anxious by day 7 of the recovery period; however, fish had recovered by day 12.

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Poster session – Thursday 4th July 2013

A9.31

Identification, characterization, and localization of soluble adenylyl cyclase in corals

Megan E Barron (Scripps Institution of Oceanography, UCSD, United States), Katie L Barott (Scripps Institution of Oceanography, UCSD, United States) and Martin Tresguerres (Scripps Institution of Oceanography, UCSD, United States)

Cyclic adenosine monophosphate (cAMP) is a ubiquitous signalling molecule. Soluble adenylyl cyclase (sAC) is a newly identified source of cAMP in diverse phyla; sAC is bicarbonate-stimulated and thus seems to be an evolutionarily conserved CO_2/pH/HCO_3^- sensor. We are investigating the presence and physiological roles of sAC in corals, where photosynthesis and calcification are sensitive to acid/base conditions. We found that corals have some of the highest known rates of cAMP production, which is further stimulated by bicarbonate, suggesting sAC activity. BLAST searches revealed two potential sAC genes in *Acropora* species. We cloned sAC cDNA from *A. yongei*, produced and purified recombinant coral sAC, and generated dose-response curves to known pharmacological inhibitors of sAC in mammals and sharks. We also generated a polyclonal antibody, which specifically recognized a ~100 kDa protein in homogenized *A. yongei* tissue. Immunofluorescence imaging revealed labelling in cells from all four coral tissue layers, with stronger signal in the aboral endoderm and the calicoblastic epithelium. Determining the properties and localization of coral sAC will contribute to a greater understanding of acid–base sensing and regulation in marine organisms. These findings may help us understand and predict responses to changes in ocean chemistry, including those due to ocean acidification.

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Poster session – Thursday 4th July 2013

A9.32

Mitochondrial complex I-capacities of notothenioid fish of different latitudinal origin in the light of ocean acidification and warming

Anneli Strobel (Alfred Wegener Institute for Polar and Marine Research, Germany), Hans O Pörtner (Alfred Wegener Institute, Germany) and Felix C Mark (Alfred Wegener Institute, Germany)

Mitochondria are a key element in shaping whole organism energy turnover, and their contribution to the special features of stenothermy and climate sensitivity in Antarctic fishes is highly relevant to the field of climate sensitivity of Antarctic ecosystems. This study elucidates whether heart mitochondria of notothenioids from different thermal regimes differ in their sensitivity to rising temperature by comparing mitochondrial capacities of the Austral notothenioid *Notothenia angustata*, the sub-Antarctic *Lepidonotothen squamifrons*, the stenotherm *Notothenia rossii*, *N. coriiceps*, and the high-Antarctic *Trematomus nicolai* and the icefish *Chionodraco hamatus*. Furthermore, we highlight the abilities of metabolic compensation for rising pCO_2 at intracellular level between Austral and Antarctic fish after acclimation to control conditions (pCO_2 0.04 kPa) and to an ambient pCO_2 of 0.2 kPa.

The scope to adjust mitochondrial oxidative phosphorylation capacities, and particularly complex I capacities, is suggested to serve as a measure for a species' plasticity to respond to rising temperature. We therefore assessed permeabilized heart fibre oxygen consumption at various respiratory states and compared complex I capacities between the different fish as a measure for their metabolic flexibility towards climate change.

Our data indicate that heart mitochondrial complex I sensitivities to acute thermal change can aid to assess if species possess high or low capacities to increase heart mitochondrial aerobic metabolism with temperature, which may become a crucial factor for the maintenance of heart performance when environmental conditions change. Our study suggests that Antarctic notothenioids have lower capacities to compensate mitochondrial oxidative capacities than their cold-temperate relatives.

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Poster session – Thursday 4th July 2013

A9.33

Linking transcriptomics to physiology – effects of chronic thermal acclimation in the Antarctic eelpout (*Pachycara brachycephalum*)

Heidrun Windisch (Alfred Wegener Institute for Polar and Marine Research, Germany), Stephan Frickenhaus (Alfred Wegener Institute for Polar and Marine Research, Germany), Uwe John (Alfred Wegener Institute for Polar and Marine Research, Germany), Rainer Knust (Alfred Wegener Institute for Polar and Marine Research, Germany), Hans-Otto Pörtner (Alfred Wegener Institute for Polar and Marine Research, Germany) and Maguns Lucassen (Alfred Wegener Institute for Polar and Marine Research, Germany)

Research on thermal biology of Antarctic marine organisms has increased awareness for their vulnerability to climate change as a flipside to their adaptation to life in the permanent cold and their limited acclimation capacities in response to varying temperatures. Within this study on the Antarctic eelpout (*Pachycara brachycephalum*), we employed a species-specific microarray of a normalized cDNA library to identify the patterns of steady-state gene expression after two months of acclimation to six temperatures between -1°C and 9°C.

Changes in cellular processes comprised signalling, post-translational modification, subcellular organization, metabolic rearrangements as well as alterations in the transcription and translation machinery. The magnitude of responses was reflected in whole animal fitness parameters. An optimal growth performance at 3°C was paralleled by a minimum in altered transcript diversity and expression levels. The up-regulation of ribosomal proteins at 5°C and above was accompanied by differential protein degradation pathways. From 7°C upwards an incipient cellular stress response became visible through an increase of transcripts for heat shock proteins and an acute inflammatory response.

Together, these patterns reflect cellular rearrangements as a consequence of cold as well as warm acclimation and/or the progressive development of functional imbalances. In the warmth, a temperature-dependent energy deficit and a shift to autophagy became visible by linking physiological performance to liver gene expression. The observed temperature-specific expression profiles revealed the molecular basis of thermal plasticity and refined the understanding of the outline and location of the thermal window of this cold-adapted species.

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Poster session – Thursday 4th July 2013

A9.34

Effects of autonomic blockade on thermal tolerance and cardioventilatory function during acute temperature challenge in rainbow trout, *Oncorhynchus mykiss*

Andreas Ekström (University of Gothenburg, Sweden), Fredrik Jutfelt (University of Gothenburg, Sweden) and Erik Sandblom (University of Gothenburg, Sweden)

Climate change models predict increased average global temperatures and more frequent extreme summer heat spells in the future. This will undoubtedly impose a significant challenge for ectothermic animals such as fish, but currently the physiological mechanisms determining the upper critical temperature (CT_{max}) are unknown. One hypothesis suggests that cardiac performance and tissue oxygen supply is the main limiting factor. While the autonomic nervous system is crucial for routine cardiac control and performance, little is known about the role of this control system during acute temperature changes.

Previous studies have shown that β-adrenergic stimulation has a cardioprotective function at critically high temperatures in fish and vagally induced bradycardia has been observed at temperatures close to CT_{max}, which may serve to enhance myocardial oxygenation, e.g. by decreasing diffusion distances and increase the residence time of the blood in the heart. Thus, given the suggested importance of cardiac function for thermal tolerance and the potential protective effects of the autonomic nervous system on the heart, we hypothesized that inhibition of the adrenergic and cholinergic components of the autonomic nervous system with sotalol (2.7 mg^{-1} kg^{-1}) and atropine sulphate (1.2 mg^{-1} kg^{-1}), respectively, would translate into changes in CT_{max}.

CT_{max} (~26°C) did not differ between treatments, although temperature-dependent cardiac performance was markedly affected. Furthermore, our data revealed an increased cholinergic tone at temperatures up to 2°C below CT_{max}, which may represent a mechanism facilitating myocardial oxygen supply at critically high temperatures in fish.

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Poster session – Thursday 4th July 2013

A10: STRESS IN THE WILD: LINKING CONSERVATION PHYSIOLOGY WITH ENDOCRINOLOGY

Organized by: Nic Bury (Kings College London), Armin Sturm (University of Stirling) and Craig Franklin (University Of Queensland)

A10.1

Rethinking the impact of chronic stressors in nature: Adaptive *versus* maladaptive outcomes

Rudy Boonstra (University of Toronto Scarborough, Canada)

The adaptations animals have in the natural world are solutions to ecological problems to which they have a long evolutionary history. The stress axis is a vital regulator of that adaptation.

Animals in nature regularly experience chronic stressors related to high population density and social pressure, increased predation risk, lack of food, severe weather, and so on. However, only some species are chronically stressed by these factors – showing chronic changes in their physiology, reproduction, and condition; others deal with such stressors acutely and then go back to the business of living.

I will present evidence that the stress axis in both groups continue to function remarkably well. The difference between chronic and acute responses of the two groups may be related to their life history. Though the biomedical literature and most of the literature on natural populations has regarded chronic stress-induced changes as pathological, I will argue they are adaptive, ultimately promoting an animal's survival and reproductive success.

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11:00 Wednesday 3rd July 2013

A10.2

Corticosterone responses and personality in birds: Individual variation and the ability to cope with environmental change

John F Cockrem (Massey University, New Zealand)

Birds can respond to an internal or external stimulus with activation of the HPA axis and secretion of corticosterone. There is considerable individual variation in corticosterone responses, and individual responses can be very different from the mean response for a group of birds.

Corticosterone responses and behavioural responses to environmental stimuli are determined by individual characteristics called personality. It is proposed that birds with low corticosterone responses and proactive personalities are likely to be more successful (have greater fitness) in constant or predictable conditions, whilst birds with reactive personalities and high corticosterone responses will be more successful in changing or unpredictable conditions.

The relationship between corticosterone responses and fitness thus depends on the prevailing environmental conditions, so birds with either low or high corticosterone responses can have the greatest fitness and be most successful, but in different situations.

It is also proposed that birds with reactive personalities and high corticosterone responses will be better able to cope with environmental changes due to climate change than birds with proactive personalities and relatively low corticosterone responses. Phenotypic plasticity in corticosterone responses can be quantified using a reaction norm approach, and reaction norms can be used to determine the degree of plasticity in corticosterone responses of individual birds, and mean levels of plasticity in responses of species of birds. Individual corticosterone responses and personality, and reaction norms for corticosterone responses, can in future be used to predict the ability of birds to cope with environmental changes due to climate change.

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11:40 Wednesday 3rd July 2013

A10.3

Corticosterone variation in feathers of shearwaters

Teresa Militão (University of Barcelona, Spain), Clare Parker (Tufts University, United States), L Michael Romero (Tufts University, United States) and Jacob González-Solís (University of Barcelona, Spain)

Feather corticosterone (CORT) analyses can indicate the level of effort invested by the bird over the previous period during the growth of the feather. Therefore, the analyses of feathers grown in different periods may help us to understand how birds allocate their effort over distinct life-history stages and how this relates to different aspects of their ecology.

In this study we analysed primary (P) and secondary (S) feathers from Cory's (*Calonectris diomedea borealis*) and Scolopi's (*C. d. diomedea*) shearwaters from both, dead birds (P2, P4, P6, P8 and S13) and birds tracked with geolocators (P1 and S13). Since feathers are moulted from the end of the breeding (P1 to P5) to the wintering period (P6 to P10 and S13), we used CORT levels to understand effort allocation between these two stages as well as relationships with breeding effort, breeding area, wintering area and sex.

Levels of CORT were greater in feathers moulted in the breeding than in the wintering period, suggesting the former is more energy demanding than the migration and non-breeding period. Additionally, CORT levels were greater for males than for females in feathers grown in the breeding period but did not differ in those grown in winter, suggesting breeding role of males is more energetically demanding than that of females. Moreover, we also detected significant differences in the CORT levels across breeding localities and wintering areas, suggesting birds are sensitive to factors, such as alien predators, distance to the foraging area or distinct traits of the wintering areas.

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12:10 Wednesday 3rd July 2013

A10.4

Effects of logging on stress hormone levels and nestling sex ratio in ovenbirds

Gary Burness (Trent University, Canada), Rhiannon Leshyk (Trent University, Canada) and Erica Nol (Trent University, Canada)

There is increasing interest in assessing the sub-lethal effects of anthropogenic disturbance on wildlife. We compared the effects of two methods of forest harvesting ('intensive' and 'typical' group-selection silviculture) on the corticosterone (CORT) levels of adult and nestling ovenbirds (*Seiurus aurocapilla*), and compared these with levels found in individuals from unharvested control sites.
We measured CORT in blood samples collected immediately after capture (adults and nestlings), and then following a standardized stressor (adults only). Adult males captured in sites subjected to intensive harvesting had higher stress-induced CORT levels, and a trend towards lower baseline levels, than individuals captured in typical and unharvested control sites. In contrast, nestlings captured in the intensive harvesting sites had higher baseline CORT levels. Interestingly, ovenbirds breeding in unharvested control sites had broods that were female biased, while logging shifted the offspring sex ratio of broods towards males.
These data suggest that intensive group-selection silviculture affects the stress axis of adult and nestling ovenbirds, and if broods of ovenbirds in undisturbed forests are usually female-biased, then the increased production of males in logged sites may impact population viability.

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12:40 Wednesday 3rd July 2013

A10.5

The effects of chronic exposure to stress associated with human disturbance on postnatal development

Hannah Watson (University of Glasgow, United Kingdom), Mark Bolton (Royal Society for the Protection of Birds, United Kingdom) and Pat Monaghan (University of Glasgow, United Kingdom)

Stressful conditions during early life can have severe consequences for postnatal development, as well as long-term consequences for fitness. While human disturbance has been widely associated with diverse behavioural and physiological effects, studies have tended to focus on adults and animals living above ground, out in the open.
Many burrow-nesting seabirds exhibit slow postnatal growth and a long chick-rearing period and, at breeding colonies exposed to tourism, nestlings can be exposed to high levels of human disturbance for long periods. Having previously shown that European storm petrels *Hydrobates pelagicus* breeding in disturbed areas suffer reduced reproductive success, we sought to develop an integrated understanding of how nestlings respond to environmental stressors associated with human disturbance.
To determine whether there was any evidence for chronic stress, baseline corticosterone (CORT) and telomere length were measured during early and late postnatal development in chicks reared in nests subject to high and low levels of visitor pressure. While both baseline CORT and telomere length declined significantly between the two time points, there was no effect of human disturbance on absolute values or the magnitude of change. However, we did find evidence for retarded growth in nestlings reared in areas subject to human disturbance. Although post-hatching survival of offspring is significantly lower in disturbed areas, there is limited evidence for effects of human disturbance on the physiological condition of young that survive to fledging.

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12:55 Wednesday 3rd July 2013

A10.6

What does a chronically stressed wild animal look like? Implications for conservation physiology

L Michael Romero (Tufts University, United States) and Molly J Dickens (University of California Berkeley, United States)

One important goal of conservation is to identify populations that have been placed at risk of extinction from anthropogenic disturbances. One proposed way to identify at-risk populations is to monitor the physiology of individual animals in order to determine whether they are chronically stressed.
Most researchers measure glucocorticoids to assess stress levels, with the common assumption that glucocorticoids will increase in chronically stressed individuals. A review of the literature, however, indicates that this assumption has little empirical support. Laboratory and field studies monitored numerous diverse species and used multiple techniques for inducing chronic stress. There was so much variation between studies that the literature does not support a generalized endocrine profile in how wild animals respond to chronic stress. The common predictions appear to be based almost entirely on theoretical models rather than empirical data.
The conclusion is that conservation physiologists cannot rely upon simple measures of glucocorticoid release to diagnose chronic stress, but instead must experimentally verify a species' specific glucocorticoid response to a specific stressor before extrapolating to free-living animals.

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14:00 Wednesday 3rd July 2013

A10.7

Non-invasive reproductive and stress endocrinology in amphibian conservation physiology

Edward Narayan (Griffith University, Australia)

The non-invasive quantification of reproductive and stress hormone metabolites in amphibians have provided field biologists with direct physiological measure of reproduction and stress with minimal disturbance to the study species. Excretory lag-time between activation of the reproductive or stress endocrine axes and appearance of the target hormone metabolites in anuran urine can be obtained using exogenous hormone challenges.
Enzyme-immunoassays were biologically validated and established for the stress hormone corticosterone and the reproductive hormones, testosterone in males; estrogen and progesterone in females. Capture handling mimics the acute stress hormone response to naturalistic stressors in anurans. Urinary corticosterone and testosterone metabolites showed similar patterns of changes (with slower lag-times) in comparison to serum hormone levels during acute capture handling of the cane toad (*Rhinella marina*). This explosive breeding species also showed increasing levels of urinary testosterone and corticosterone during exposure to a moderate capture and handling stressor.
Species differences in baseline corticosterone and short-term corticosterone stress responses were demonstrated in mainland *versus* small island sub-populations of the *Platymantis* sp. Baseline urinary corticosterone was also positively associated with prevalence of the pathogenic chytrid fungus (Bd) in adults of the Stony Creek frog (*Litoria wilcoxii*). Baseline levels of urinary corticosterone were significantly different between lowland and highland sub-populations of the Great barred frog (*Mixophyes fasciolatus*) in South East Queensland, Australia.
This invited paper discusses the strengths, limitations and provides future research directions that will increase data reliability and value of non-invasive endocrinology in amphibian conservation physiology.

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14:40 Wednesday 3rd July 2013

A10.8

Psychotropic drugs as behavioural disruptors in a non-target marine organism: What does the cuttlefish on Prozac tell us?

Carole Di Poi (Normandie Université GMPc, France), Anne-Sophie Darmaillacq (Normandie Université GMPc, France), Michel Boulouard (Normandie Université GMPc, France) and Cécile Bellanger (Normandie Université GMPc, France)

Early life stages of the cuttlefish, *Sepia officinalis*, occur in coastal marine waters, chronically contaminated by xenobiotic agents including pharmaceuticals. Hatchlings do not benefit from parental care, so they need to cope autonomously with ecological demands such as foraging and predator avoidance. This period is a critical window during which animals experience behavioural and neural maturation driving to a high sensitivity to exogenous stimuli. The presence of antidepressants in the environment has the potential to generate neural disrupting effects, yet their putative effects on behaviour have not received much attention. The study aimed to evaluate the effects of acute exposures to fluoxetine (FLX, Prozac), a selective serotonin reuptake inhibitor, on ecologically relevant behaviours in newly hatched cuttlefish. Seven day-old animals were exposed at concentrations ranging from 0.05 to 1 $mg.L^{-1}$ for 5 hours before testing the efficiency of their predatory (prey-capture success, locomotor activity) and antipredator (body patterning) behaviours. A dose-dependent effect of FLX was observed on the mortality rate. FLX also decreased significantly the prey-capture success and increased the basal activity of animals at 0.5 $mg.L^{-1}$; but, it did not alter the camouflage efficiency. The study shows the interplay between environmental contaminants and animal physiology in regulating the expression of behaviour; but the doses tested are 1,000-fold higher than environmental concentrations. It brings indirect evidence of the role of the serotonergic system in the neural control of feeding and locomotor behaviours in cuttlefish, as well as provides a starting point for toxicological assessment of FLX in a non-target organism.

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15:10 Wednesday 3rd July 2013

10.9

Divergent stress responses and behaviour in early and late emerging Atlantic salmon (*Salmo salar*)

Svante Winberg (Uppsala University, Sweden), Per-Ove Thörnqvist (Uppsala University, Sweden), Martin H Larsen (Danish Technical University, Denmark), David Hammenstig (Gothenburg University, Sweden), Jörgen Johnsson (Gothenburg University, Sweden), Kim Arestrup (Danish Technical University, Denmark) and Erik Höglund (Danish Technical University, Sweden)

Behavioural plasticity appears to be related to stress coping styles. Animals displaying a proactive coping style easily develop behavioural routines whereas those showing a reactive coping style are more responsive to environmental stimuli and do not develop routines. In addition to behavioural plasticity, a proactive stress coping style is characterised by aggressive behaviour, high sympathetic but low HPI/HPA axis reactivity.

The hatchery environment being highly stable and predictable may select for a proactive phenotype. This may be one reason why fish raised in captivity often have difficulties adapting to wild conditions. In salmonids, time of emergence appear to be related to stress coping style, early emerging fry being proactive and late emerging fry reactive.

In a series of experiments salmon were sorted for early and late emergence. When salmon were exposed to confinement differences in brain gene expression between the early and late emerging fraction were found. Genes linked to aggression such as GABA-RAP, ependymin, and $5HT_{1A}$, point towards a reduction of aggression in late emerging salmon. Boldness and behavioural response towards hypoxia also differed between the early and late fraction. Differences in behaviour, survival and growth rate between the early- and late-emerging salmon will be discussed.

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16:15 Wednesday 3rd July 2013

A10.10

Effects of maternal oxidative stress and gametic cortisol exposure on sockeye salmon offspring

Jessica J Taylor (Carleton University, Canada), Natalie M Sopinka (University of British Columbia, Canada), Samantha M Wilson (Carleton University, Canada), William G Willmore (Carleton University, Canada), Scott G Hinch (University of British Columbia, Canada), David Patterson (Fisheries and Oceans Canada, Canada) and Steven J Cooke (Carleton University, Canada)

As adults, sockeye salmon (*Oncorhynchus nerka*) migrate from ocean feeding grounds to their natal freshwater stream to spawn, along the way encountering a variety of stressors. Much research has focused on how adult sockeye salmon physiology and survival are influenced by various stressors, however, the extent to which offspring experience oxidative stress based on stressors experienced by their parents has not been studied. Oxidative stress occurs when there is an imbalance in the production of free radicals and their absorption, and can increase due to physiological stress. This imbalance can damage DNA, proteins and lipids, which are subsequently deposited into eggs during oogenesis. The objective of this study was to determine the transgenerational oxidative stress effect of both maternal and oxidative stresses that occur during the migration in sockeye salmon whose life history is a delicate balance of resource allocation. Samples of plasma, liver, heart, brain and gametes were collected from female sockeye salmon, as well as male gametes for fertilization, at the end of the freshwater migration route (Harrison River spawning grounds, Canada). Immediately post fertilization, some of the offspring were exposed to 1,000 ng/ml cortisol to simulate maternal stress. Tissues and offspring at pre-fertilization, 24 hours post fertilization, eyed, hatch and emergence were then assayed for antioxidant capacity as well as for oxidative DNA damage. Results of the study will indicate whether factors involved in oxidative stress (damage, protection) encountered during salmon migration are passed from adult to offspring at the spawning grounds.

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16:45 Wednesday 3rd July 2013

A10.11

The behavioural response to waterborne chemical cues by coral reef fish larvae may impact the effectiveness of marine protected areas

Danielle L Dixson (Georgia Institute of Technology, United States) and Mark E Hay (Georgia Institute of Technology, United States)

Recent studies document larvae of coral reef fishes recruiting to home reefs on which they were spawned, despite long pelagic larval durations. This suggests larval behaviour may influence settlement site selection, and that larvae are not delivered to reefs as passive particles but choose reefs. Larvae may use chemical cues from the benthic environment to identify and selectively recruit to desirable reefs. We tested the behavioural response of 15 species of juvenile reef fishes to waterborne chemical stimuli associated with water from 3 paired sites in Fiji, each containing coral-dominated marine protected areas (MPAs) and adjacent seaweed-dominated fished non-MPAs. Results from choice flume assays revealed that all fishes strongly preferred MPA to non-MPA odours. However, fish started to avoid MPA odours if additional odours from common blooming seaweeds (e.g. *Sargassum*, *Turbinaria*) were added; likewise, previously non-preferred waters became attractive when odours from *Acropora* corals were added, suggesting that fishes are using the presence of specific benthic species to assess habitat desirability. Field transects assessing recruitment confirmed the disjunction between the MPA and non-MPA locations; MPAs had significantly more newly-settled recruits across all species despite having more predators than non-MPA sites. Strong selection for larvae choice of settlement sites is not surprising, given the fitness consequences of habitat choice. However, if larval fish are programmed to reject degraded habitats based on odour cues alone, conservation efforts using MPAs intended to produce larvae to rescue damaged reefs will be short-circuited.

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17:00 Wednesday 3rd July 2013

A10.12

Mothers may know best, even when stressed: Intergenerational effects of stress in sockeye salmon

Natalie M Sopinka (University of British Columbia [UBC], Canada), Scott G Hinch (UBC, Canada), Collin T Middleton (UBC, Canada), Jayme A Hills (Fisheries and Oceans Canada, Canada), Stephen J Healy (UBC, Canada), Melissa A Lesko (UBC, Canada) and David A Patterson (Fisheries and Oceans Canada, Canada)

Parental stress is known to compromise offspring quality in vertebrate taxa, but little is known about such effects in fish with unique reproductive life histories. In British Columbia, Fraser River sockeye salmon (*Oncorhynchus nerka*) complete rigorous migrations from the Pacific Ocean to reach natal freshwater areas to reproduce then die. Throughout their spawning migration, sockeye salmon encounter numerous anthropogenic and environmental stressors known to influence adult reproduction. Reproductive impairment may have resonating effects on the next generation and contribute to recent population declines of this iconic natural resource. To examine the intergenerational effects of stress, upriver migrating adults were caught, held in captivity and subjected to a stressful chase event twice a day for 6 weeks until maturation, which corresponded with peak spawning of fish in the wild. Offspring reared from chased mothers had lower survival and poorer body condition. Interestingly, these same offspring had superior swim performance, more streamlined bodies, larger fins and larger eyes. Behavioural responses in the threat of predation were assessed, and egg cortisol levels were quantified to explore the physiological mechanisms driving the observed intergenerational effects. Collectively, the findings provide evidence that parental stress in sockeye salmon has consequences, but that stressed mothers may prime their young with traits to enhance survival in a challenging environment.

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17:15 Wednesday 3rd July 2013

A10.13

Liver histopathology in native fish from Northwestern Portuguese rivers – preliminary results

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The assessment of water quality involving fish biomarkers, namely histopathology is a useful tool in biomonitoring programs. Therefore in 2011, trout (*Salmo trutta*), barbel (*Luciobarbus bocagei*), chub (*Squalius carolitertii*) and nase (*Pseudochondrostoma duriensis*) were electrofished in order to evaluate fish liver histopathology. The nine stretches studied (from Ave, Cávado, Douro and Leça rivers) presented different ecological status. The main histopathological changes observed were: lymphocytic foci (LF), macrophage aggregates (MA), parasitic granuloma (PG), unspecific granuloma (UG), inflammatory foci (IF), vacuolation (Va), necrosis (Ne) and picnotic cells foci (PCF). All species presented high prevalence of the two main lesions observed: LF and Ne. Trout showed the lowest number of different lesions. MA prevalence was high in all species except in trout. PG were not observed in nase from locations with a good ecological status. In both surveys nase presented the highest grades of LF, PG, UG, IF and PCF. In the same species the locals with worst water quality showed highest MA severity grades. The Ne severity in chub exhibited a little increase with the water quality degradation. These preliminary data reflect some variations between liver lesions and water ecological status, which is probably, connected with low chemical pollution levels. This is in accordance with the lack of any observation of gross lesions and (pre)neoplasic liver lesions. Moreover, PG is the best related liver lesion with water ecological status. Supported by FCT project PTDC/BIA-ECS/114859/2009, programme COMPETE and FEDER Funds.

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Poster session – Thursday 4th July 2013

A10.14

Does marine pollution by psychotropic drugs impact the chance of survival in young cuttlefish (*Sepia officinalis*)? A neuro-behavioural study

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Embryonic and early postembryonic development of cuttlefish *Sepia officinalis* occurs in marine coastal waters. These stages are sensitive periods for the development of complex behaviours and learning abilities. Among chemicals continuously released into aquatic environment, pharmaceutical products may pose a long-term risk to cuttlefish survival by disrupting the setting-up of these abilities. Considering the role played by serotonin in nervous system development and on the regulation of a wide range of behaviours (feeding, locomotion, etc), this study focused on fluoxetine (FLX, Prozac®), a selective serotonin reuptake inhibitor (SSRI, antidepressant).The goal was to determine the effects of subchronic waterborne FLX exposure during embryogenesis on the expression of fundamental behaviours and the maturation of the brain in cuttlefish. Fertilized cuttlefish eggs were exposed to FLX concentrations (1, 10 $\varepsilon g.L^{-1}$) in a flow-through system during the last 15 days of embryonic development. Locomotor activity and camouflage efficiency of hatchlings were investigated. A volumetric study estimated the size of brain structures known to be implicated in complex behaviours. In the same brain structures, cell proliferation was assessed using the endogenous mitotic marker phosphorylated histone H3. Preliminary results showed FLX exposure tends to alter defensive behaviours of hatchlings. The brain maturation analysis (in progress) will determine whether FLX exposure has neurobiological consequences and might explain the behavioural changes observed. Such modifications on brain and behaviour development could have important population-level consequences.

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Poster session – Thursday 4th July 2013

A10.15

Oxidative stress in fish gill and liver use in prediction of Water Framework Directive ecological status classification

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Anthropogenic activities of waste can modify the chemical composition of natural aquatic environments. Fish are generally considered optimal model organisms for monitoring aquatic environments. The fish gill is involved in vital functions like respiration, osmoregulation, acid–base balance and nitrogenous waste excretion and is the first organ to be in contact with xenobiotics. The assessment of hepatic defence and damage responses is also essential, due to the important role of the liver on many vital functions, e.g. accumulation, biotransformation and excretion of contaminants.
In this work, biochemical responses (lipid peroxidation [LP], glutathione-S-transferase [GST], glutathione peroxidase [GPx], superoxide dismutase [SOD] and catalase [CAT]) were assessed in the gill and liver of three fish species (trout [*Salmo trutta*], barbel [*Luciobarbus bocagei*] and nase [*Pseudochondrostoma duriense*]) captured, during summer and winter in nine Northwestern Portuguese rivers locations with previous ecological status classification, to compare and determine which tissue and responses better reflect the ecological status classification (good, poor and bad) using discriminant analysis. Results showed gill responses in general discriminated ecological status; however, the combined use of the two organs' responses allowed better discrimination, indicating a better approach. In winter, gill and liver responses used only allow us to discriminate locations classified with good ecological status. In general, gill LP levels, gill GST and gill and liver GPx activities were the responses that allowed better discrimination of the locations according to ecological status classification.

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Poster session – Thursday 4th July 2013

A11: GENERAL ANIMAL BIOLOGY

Organized by: Jonathan Stecyk (University of Alaska Anchorage)

A11.1

Aerobic capacities and seasonal differences in diving behaviour of Weddell seals

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Adult Weddell seals (*Leptonychotes weddellii*) haul-out on the ice in Oct/Nov for the breeding season and remain relatively inactive for ~4 months until their moult in Jan/Feb. To determine whether this reduced activity during the austral summer constrains diving abilities, this study compared the physiology of 48 pre-breeding females in Oct/Nov to 53 post-moult female Weddell seals in Jan/Feb in McMurdo Sound, Antarctica.

Seals handled in Oct/Nov were larger and in better condition, with greater lipid stores and blubber depths (p<0.001). Despite having lost mass and lipid stores across the breeding and moulting periods, there was no seasonal difference in stress hormone levels (cortisol, 83.7±1.4 µg/dL, p=0.736). Animals had significantly higher blood O_2 stores (66.0±1.9 vs. 61.0±1.7 mL O_2/kg lean tissue, p=0.009) and slightly higher myoglobin concentrations (90.4±2.1 vs. 86.9±3.1 mg/g tissue, p=0.350) and total body O_2 stores (124.3±2.2 vs. 117.0±2.3 mL O_2/kg lean tissue, p=0.065) at the end of the moult. In contrast, anaerobic capacity, as measured by muscle lactate dehydrogenase activity was significantly lower following the moult (505.8±16.2 vs. 653.9±27.8 IU/g wet tissue, p<0.001), but positively correlated with body size. In the eight weeks following the moult, larger animals and those in better condition (%lipid), made significantly longer but fewer dives when compared with smaller individuals (p<0.001, n=52 seals).

The preservation of O_2 stores in the face of reduced activity is likely critical for the maintenance of foraging capabilities, so that following the moult Weddell seals can rapidly regain body mass lost during the summer period.

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13:20 Friday 5th July 2013

A11.2

The energy expenditure of walking or scaling of terrestrial transport costs in animals, and then a twist

Lewis G Halsey (University of Roehampton, United Kingdom), Craig R White (University of Queensland, United Kingdom) and Rory P Wilson (Swansea University, United Kingdom)

We report two independent studies that in combination provide insight into terrestrial transport costs.

The first study compiled measurements of the net cost of pedestrian transport (NCOT; ml O_2 m^{-1} kg^{-1}) for $2O_2$ species spanning over nine orders of magnitude in mass from fruit flies to Asian elephants. When analysed using a phylogenetic approach, NCOT varied only as a function of mass (M, kg; NCOT = 0.59M–0.27 [95%CI: -0.30 to -0.25]) and did not differ systematically among lineages (insects, crustaceans, arachnids, amphibians, reptiles, birds, mammals); or among polypeds, quadrupeds, and bipeds; and did not differ between endotherms and ectotherms. This suggests that the fundamental physics of moving, i.e. the need to exert forces on the substrate and to support and intermittently raise the body, is the universal, main factor describing terrestrial transport costs.

The second study measured the gross cost of pedestrian transport (GCOT; ml O_2 min^{-1} kg^{-1}) of turning while walking, using humans as a model. The effect on GCOT of turning was substantial and can be described as a function of turn angle (θ, °; GCOT = 0.115θ + 17.6), indicating that consideration of only straight-line transport risks considerably underestimating transport costs. The energetic efficiency of straight-line travel implies that search strategies should favour constrained turn angles unless the potential benefits of turning offset the cost. Yet variable energetic costs are not incorporated into optimal search models.

Further important work linking these two studies would describe how turning costs scale with straight-line travel speed and straight-line travel costs.

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13:30 Friday 5th July 2013

A11.3

The interaction effects of gravidity, digestion, and exhaustive activity in the viviparous checkered garter snake

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The goal of this study was to determine how various physiological states, individually and combined, influence oxygen consumption (VO_2) in the viviparous snake (*Thamnophis marcianus*). Specifically, VO_2 during gravidity, combined with digestion, activity, and/or post-prandial activity was measured.

We determined whether this species demonstrates an overall VO_2 that was additive (simple summation of the oxygen demands for each physiological state) or was prioritized (oxygen delivery for one state was prioritized over other states; Bennett & Hicks, 2001). We predicted that gravid females would have a significantly elevated VO_2 over non-gravid females, and that there would be a summation effect of gravidity with digestion, activity, and post-prandial activity.

To test our hypothesis, we measured VO_2 using an oxygen analyser in a flow-through respirometry system. Gravid and non-gravid female VO_2 values were measured during rest, exhaustive activity, digestion, and post-prandial exhaustive activity. Gravidity and the degree of embryonic development, was determined using a portable veterinary ultrasound machine.

Results indicate that resting VO_2 during gestation remains elevated by 1.68–3.40X over resting non-gravid values. When combining gravidity with activity, a prioritization effect is realized, while the combination of gravidity and digestion results in a more-than-additive VO_2 response. In both gravid and non-gravid females, post-prandial activity results in significantly higher peak VO_2 than either digestion or activity alone (1.2X higher). This value is significantly lower than expected, and is indicative of a prioritization response regardless of reproductive state.

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13:40 Friday 5th July 2013

A11.4

Physiology and behaviour of lactating laboratory mice able to cool down while resting away from their pups

Teresa G Valencak (Veterinary University Vienna, Austria) and John R Speakman (University of Aberdeen, United Kingdom)

According to the heat dissipation limitation hypothesis lactating females are constrained by their capacity to get rid of excess metabolic heat. However, previous experiments supporting this hypothesis have confounded the impact of temperature on the mothers with the impact on the pups. We aimed to separate these effects in laboratory mice from the MF1 strain that had access to cages at two ambient temperatures (10 and 21°C) joined by a tube.
Food was available only in the cold cage, but females could choose go to this cage to cool down while pups were housed in the warmer cage. Control animals had access to the same cages both maintained at 21°C. We hypothesized that if females were limited by heat dissipation, alleviating the heat load by providing a cool environment close to the nest would allow them to dissipate more heat, intake more food, generate more milk and hence wean heavier litters. We thus measured maternal energy budgets, monitored time courses of core body temperature and physical activity. To minimize the variance in energy budgets all litters were adjusted to 12 (±1) pups.
Females in the experimental group had higher energy intake (F_{1},14=15.8; p=0.0014), higher assimilated energy (F_{1},13=10.7; p=0.006) and provided their pups with more milk (F_{1},13=6.65; p=0.03). Yet, mean pup growth rates were similar (F_{1},13=0.06; p=0.8). Our data emphasise the difficulties of separating heat dissipation limits (HDL) from changes in pup demand and the problems of inferring milk production indirectly from pup growth.

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13:50 Friday 5th July 2013

A11.5

The effect of costs of thermoregulation on daily energy expenditures and winter mortality in root vole *Microtus oeconomus*

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It has been hypothesized that individuals with high daily energy expenditures (DEEs) may have higher overwinter mortality rates due to inability to balance energy budgets. To test this we increased thermoregulatory costs in free-ranging root voles by shaving their backs and monitored their mortality in two consecutive winter seasons.
Shaving had no appreciable effect on survival. The effect of extra heat loss on DEE was significant and also manifested as shaving x body mass interaction. The DEE of small shaved individuals tended to be higher than larger ones, probably due to higher surface-to-volume ratio.
We also carried out laboratory experiment on shaved voles kept at 5°C. We found no significant effect of shaving on locomotor activity, and no effect on energy assimilation rate. Taken together, our results suggest strong flexibility of energy budgets, which enabled shaved individuals to ameliorate increased costs of thermoregulation.

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14:00 Friday 5th July 2013

A11.6

Metabolic transition during feast and famine in the tarantula *Acanthoscurria geniculata*

Johannes Overgaard (Aarhus University, Denmark), Peter S Knudsen (Aarhus University, Denmark), Hans Malte (Aarhus University, Denmark) and Tobias Wang (Aarhus University, Denmark)

Spiders are opportunistic feeders that may experience fluctuating food availability. To tolerate prolonged fasting, spiders must therefore be able to balance the use of endogenous energy stores with metabolism, while retaining the capacity to subdue large prey and quickly up-regulate digestive functions. Here we studied the effects of prolonged fasting on metabolic rate, the magnitude and duration of the metabolic response to digestion (specific dynamic action, SDA response) as well as changes in bodily concentration of lipid and protein during fasting and immediately after feeding in the Brazilian white knee tarantula.
Our study reveals several physiological 'records' including longest recorded fasting period and highest factorial scope of an SDA response, suggesting that these spiders are eminently adapted to prolonged fasting and gorge feeding. Over the period of 550 days of fasting, metabolic rate fell to a third of the initial values. Much of this depression was related to the loss of dry mass since metabolic rate per g dry mass decreases less. Thus, long-term starvation is associated with an uptake of water and we speculate that this response is linked to preservation of heamolymph pressure to maintain proper motor functions. Feeding on rat pups resulted in an almost 30-fold increase in oxygen uptake over several days. The peak of the SDA response was higher and the duration shorter in long-term starved spiders (>200 days) than in short-term fasted spiders (~25 days) illustrating that the ability to handle huge meals is preserved during long term starvation.

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14:10 Friday 5th July 2013

A11.7

The effects of compensatory growth on aerobic scope and temperature preference in European minnows

Shaun S Killen (University of Glasgow, United Kingdom) and Neil B Metcalfe (University of Glasgow, United Kingdom)

Compensatory growth – the rapid growth that occurs after a period of reduced feeding – can have lasting adverse effects locomotor performance, but influences on other behaviours and physiological traits are not well-understood. We examined the effects on temperature preference and aerobic scope in individual European minnows *Phoxinus phoxinus*.
Fish were either fed a sub-maintenance ration for three weeks during the early juvenile stage or fed *ad libitum* during this time. After an additional 11 weeks of *ad libitum* feeding in both treatments, fish in the food-deprived treatment were found to have faster growth rates as compared to control fish, and tended to have an elevated standard metabolic rate and reduced aerobic scope. Interestingly, growth-compensated individuals tended to prefer warmer temperatures when presented with the opportunity to select their preferred thermal environment.
Given adequate food-availability, a warmer environment could allow growth-compensated individuals to maximize growth potential. It is also possible that altered growth trajectories caused by early nutritional deficits may cause the optimal thermal range of individuals to shifts toward warmer temperatures. In the wild, preference for warmer temperatures among individual freshwater fish could translate to remaining along stream shorelines or side-pools, which could represent an increased risk of predation by terrestrial and aerial predators.

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14:20 Friday 5th July 2013

A11.8

Swimming performance of juvenile yellowtail kingfish (*Seriola lalandi*)

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Swimming exercise at optimal speed may represent a natural, non-invasive and economical approach to improve health, welfare, growth and filet quality of yellowtail kingfish in recirculating aquaculture systems (RAS). In order to implement an optimal exercise regime in RAS we have quantified the swimming performance (optimal swimming speeds [U_{opt}] and costs of transport [COT]) in Blazka-type swim-tunnels (test range 0.2–1.0 m s^{-1} with step-wise increments of 0.2 m s^{-1} at one-hour intervals) of three size-classes of juveniles:
(1) 145±4 mm total body-length BL, 34 Ψ 3 g body-weight BW;
(2) 252±7 mm BL, 206 Ψ 14 g BW; and
(3) 311 ± 5 mm BL, 392 Ψ 16 g BW.
Blood was sampled immediately after swimming, hematocrit was determined (48–49% for all groups) and plasma was used for measurements. For the three size-classes we found U_{opt} of (1) 0.70 m s^{-1} or 4.83 BL s^{-1}, (2) 0.82 m s^{-1} or 3.25 BL s^{-1} and (3) 0.85 m s^{-1} or 2.73 BL s^{-1}.
Combined with data from literature on U_{opt} for larger fish, we have established a relation between BL and swimming speed in BL s^{-1} of y = 234.07 x -0.779 (r^2 = 0.9909). Significantly higher lactate levels and osmolality, and trends of lower levels of cortisol, NEFA and glucose, in fish that were able to swim at highest speeds *versus* those that fatigued suggest that high performance is reflected by the ability to cope with (and not to get hyperstimulated by) increased metabolic demands and metabolite production.

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14:30 Friday 5th July 2013

A11.9

Living life on the edge: Unique nano-structures on the guillemot eggshell assist with nesting in a harsh environment

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Among vertebrates, the avian egg is unique in its structure and form. Bird eggs show a large range in shape, size and colour, the diversity of which has inspired much interest and debate. We have identified a new biological structure present in the surface of the eggshell, which is seemingly specific to one group of birds whose breeding ecology is unique within the avian kingdom.
The guillemot group (*Uria* sp.) of the Alcidae family breed on exposed marine cliff faces, and with no nest used, the eggs are laid directly on the rock surface. The eggs are constantly exposed to sea-salt spray and guano from the incubating parents. The conical-like structural protrusions present in the eggshell surface of guillemot eggs are analogous with the nano-scale hierarchical arrangements identified in lotus leaves (*Nelumbo* sp.), the purpose of which is self cleaning. These structures are entirely absent in the eggs of 217 other birds species studied, including those nesting in similar environments, and those closely related to the guillemots from a phylogenetic perspective.
Through comparative experiments we demonstrate how the guillemot eggshells exhibit hydrophobicity, have increased surface roughness and surface area, and a high rate of gas exchange under constant conditions. We conclude that these differences, a result of the conical protrusions in the eggshell surface, are an adaptation to the unusual breeding environment of the guillemot, in particular the salt and guana exposure.

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14:40 Friday 5th July 2013

A11.10

Back in black: New evidence on the colour and nature of the isolated *Archaeopteryx* feather

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Archaeopteryx has been regarded as an icon of evolution ever since its discovery from the Late Jurassic limestone deposits of Solnhofen, Germany in 1861. The mosaic of plesiomorphic and derived anatomical traits in these fossils has inspired a rich scientific literature on *Archaeopteryx* and the origin of birds, yet the animal's colour, a diverse and multifunctional trait in modern birds, has remained only speculative. Additionally, unresolved questions have persisted regarding the anatomical identity and composition of the isolated feather specimen, which was the first described *Archaeopteryx* fossil and the only one preserved as a dark trace.
Here we report the first evidence of colour from *Archaeopteryx*, based on scanning electron microscopy and energy-dispersive X-ray analyses that reveal the presence of fossilized colour-imparting melanosomes in the isolated feather (MB.Av.100). Using a phylogenetically diverse database of 115 extant bird feathers (representing 87 taxa from 27 orders), quadratic discriminant analysis of five properties of melanosome morphology predicts that the original colour of the *Archaeopteryx* feather was black, with 95% probability. Furthermore, re-examination of the feather's morphology leads us to interpret it as an upper major primary covert, contrary to previous interpretations. Additional findings reveal that the specimen is preserved as an organosulphur residue, and that barbule microstructure identical to that of modern bird feathers had evolved as early as the Jurassic. As in extant birds, the extensive melanization would have also provided structural advantages to the *Archaeopteryx* wing feather during this early evolutionary stage of dinosaur flight.

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14:50 Friday 5th July 2013

A11.11

Structure and properties of the ultra-black skin colouration of the West African Gaboon viper (*Bitis rhinoceros*)

Marlene Spinner (Kiel University, Germany), Alexander Kovalev (Kiel University, Germany), Guido Westhoff (University of Bonn, Germany) and Stanislav N Gorb (Kiel University, Germany)

The West African Gaboon viper (*Bitis rhinoceros*) is well camouflaged due to the contrasting geometrical colouration pattern of pale and black markings on its back. The velvety surface texture of the black skin regions purports a spatial depth imitating the variations of light and shade on the rain forest ground. In our study, for the first time, we investigated the micromorphology, optical properties, and physical principles behind this exceptional ultra-black appearance.
In addition to different content of dark pigments in the scale material, black scale surfaces have a unique hierarchical pattern of leaf-like microstructures covered with nano-ridges, which were not found on the pale scales. Ultra-black scales have a five times lower reflectivity and higher absorbance than other scales for the UV to near-IR spectral range and reflect less than 5% of the light reflected by a PTFE diffuse reflectance standard in any direction.
Comparison between our experimental data and the Oren-Nayar reflectance model, and the preservation of black colouration on black scales, sputter-coated with a thin layer of metal, provided clear evidence that the presence of nanostructures is crucial to the ultra-black appearance of the snake scales.

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15:00 Friday 5th July 2013

A11.12

Why do insects enter chill coma? Low temperature and high extracellular potassium compromises muscle function in *Locusta migratoria*

Anders Findsen (Aarhus University Denmark, Denmark), Thomas H Pedersen (Aarhus University, Denmark), Ole B Nielsen (Aarhus University, Denmark) and Johannes Overgaard (Aarhus University, Denmark)

Many insect species enter a comatose state when their body temperature is lowered to a critical limit (critical thermal minimum) but the physiological and cellular processes that underlie chill coma are still unresolved. Several studies have demonstrated transition into chill-coma involves a disruption of neuromuscular performance and that ion homeostasis (particularly extracellular [K^+]) is disrupted during chill coma. Neuromuscular failure associated with chill coma is therefore likely to be caused by direct effects of reduced temperature as well as indirect, force-depressing effects that occur secondary to loss of ion-homeostasis. Using isolated tibial muscle of the chill susceptible locust we investigated the relationship between tetanic force production, temperature and extracellular [K^+]. Tetanic force was elicited every 10 minutes using 2 s trains of 1 ms 12-V pulses and a frequency of 60 Hz. To explore to what extent loss of force during cold exposure reflects loss of muscle function, all experiments were performed both in the presence and absence of TTX, which selectively inhibits motor nerve function in insects. Maximum tetanic force decreased approximately 75% when temperature was reduced from 23°C to 0.5°C. Similarly, elevation of extracellular [K^+] from a control value of 10Ψ30 mM also caused a 50% reduction of force. When muscles were simultaneously cooled and exposed to elevated [K^+], force was reduced to around 1% of the control. These results suggest that both temperature and ion disruption are of importance when animals enter chill coma.

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15:40 Friday 5th July 2013

A11.13

Thermoregulatory strategy shifts with development in harp seal pups (*Pagophilus groenlandicus*)

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Mammals must balance heat production and loss to maintain thermoregulatory homeostasis. Animals born in polar regions face additional challenges because extreme environmental conditions and small body size contribute to heat loss. Harp seals (*Pagophilus groenlandicus*) are born on pack ice, with little blubber, and a wettable lanugo coat, but 12 days later weaned pups have a developed blubber layer and begin to moult. To determine if thermoregulatory capability and strategy change as pups develop, we examined five age classes of harp seal from birth to post-moult. We measured insulative capacity through percentage blubber, blubber and pelt thermal conductivity, and thermal resistance. We assessed potential for additional heat generation by non-shivering thermogenesis (NST), through uncoupling protein 1 (UCP1) expression, and mitochondrial density in brown adipose tissue (BAT). Blubber volume significantly increased (p<0.001), though there was no significant difference in blubber conductivity across age classes (p=0.969). Pelt conductivity did not differ except in nine-day old pups, which had higher conductivity (p<0.001). Overall thermal resistance was not different among ages (p=0.948), but the contribution of blubber increased from 17.5±0.03% of resistance in neonates to 75.87±0.01% of resistance after three weeks. While BAT of younger pups expressed UCP1, expression and mitochondrial density quickly declined, and by weaning, the ability to produce heat via NST was lost. UCP1 expression was negatively correlated with increasing percentage of blubber across age classes (r^2=0.756, p=0.001). These findings suggest additional thermogenesis is no longer necessary when blubber, rather than wettable fur, is the main thermal barrier.

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15:50 Friday 5th July 2013

A11.14

Water relations of three *Ceratitis* species with regard to temperature and relative humidity

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Fruit flies (Diptera: Tephritidae) represent the most significant pests of horticulture globally. Little research has been undertaken to determine the desiccation resistance and water loss of these pests despite its value for modelling their distribution, abundance and invasive potential. We determined the effect of the interaction of temperature (25 and 30°C) and relative humidity (0, 33, 75 and 100% RH) on the survival time and water loss rate of the three most important fruit fly pests of southern Africa: *Ceratitis capitata*, *C. cosyra* and *C. rosa*. Mean body water content varied between species (*C. capitata* = 5.760.1 mg, *C. cosyra* = 6.0±0.2 mg, *C. rosa* = 6.4±0.2 mg) and in all three species the body water content of females was higher than that of males. Mean survival time of *C. rosa* (47±1 hours) was significantly lower than that of the other two species (*C. capitata* = 51±2 hours, *C. cosyra* = 50±1 hours). In all species, survival time declined with increasing temperature and increased with increasing RH. The interaction of species, sex, temperature and relative humidity affected water loss rate. When held at 0% RH, temperature had a pronounced effect on water loss rate in *C. cosyra* and *C. rosa*, but not in *C. capitata.* However, when held at high humidity and particularly at 30°C, the water loss rate of *C. capitata* was high relative to *C. cosyra* and *C. rosa*. Quantification of protein, carbohydrate and lipid reserves supported these observations of whole-animal variation in water loss rates.

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16:00 Friday 5th July 2013

A11.15

UCP1-mediated non-shivering thermogenesis does not underlie the evolution of cold-tolerance in multiple eutherian lineages

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Uncoupling protein 1 (UCP1) is critical for the nonshivering thermogenic (NST) function of brown adipose tissue (BAT). BAT is recognized to play a key thermoregulatory role in small-bodied and hibernating eutherian mammals, and is considered to be especially important for neonates of cold-tolerant species. In support of this conclusion, UCP1 pseudogenization has only been described in one mammalian lineage, pigs, whose young are notoriously poor thermoregulators.

To investigate if *UCP1* non-functionalization is also associated with cold-intolerance in other placental mammal lineages, we conducted a phylogenetic survey of *UCP1* across 67 eutherian species and reveal independent losses of *UCP1* function in the ancestors of paenungulates, xenarthrans, delphinids and equids. Our findings are corroborated by a near neutral pattern of evolution in these loci and absence of discernible BAT in neonates of each of these lineages. While *UCP1* inactivation is correlated with cold-intolerance in xenarthrans and manatees, disrupted *UCP1* loci in killer whales, horses, woolly mammoth and Steller's sea cow indicate that cold-tolerance is not associated with BAT-mediated NST in these lineages.

Our findings shed light on the potential energetic costs of BAT and question the thermogenic importance of *UCP1* during eutherian evolution.

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16:10 Friday 5th July 2013

A11.16

Contaminant-specific targeting of olfactory sensory neuron classes: Connecting neuron class impairment with behavioural deficits

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Olfactory sensory neurons (OSNs), along with support cells, make up the olfactory epithelium of fish. There are three known OSN cell types; cilated, microvillous, and crypt cells, all of which can be bound and activated by odourants in the surrounding water. Specific odourants have been shown to activate one or multiple classes of OSNs. Microvillous cells have been shown to be the only OSN class to respond to L-alanine, while ciliated cells are the only OSN class that responds to taurocholic acid. By measuring the response of the olfactory epithelium to these two odourants, we were able to measure how exposure to nickel or copper specifically affected the function of microvillous or ciliated cells, respectively.

The results demonstrated that in both a lab-reared population of fathead minnows and wild yellow perch ciliated cells were susceptible to copper but not nickel, while microvillous cells were susceptible to nickel but not copper. These effects were compared to the effect of copper or nickel on the response of fathead minnows to a conspecific antipredator cue. Fathead minnows exposed to copper were unable to respond to the cue, while those exposed to nickel were able to respond to the cue. These results demonstrate that the response of fathead minnows to a conspecific antipredator cue is dependent on ciliated, but not microvillous OSNs.

Our work is the first to demonstrate OSN-specific effects of contaminants which can impair chemosensory-induced antipredator behaviour mediated by individual OSN classes.

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16:20 Friday 5th July 2013

A11.17

Trophic contamination by pyrolytic and petrogenic polycyclic aromatic hydrocarbons: Effects on metabolic and swimming performance in zebrafish (*Danio rerio*)

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Polycyclic aromatic hydrocarbons (PAH) are one of the most widespread organic pollutants. Depending on their origins, PAH are characterized by different chemical properties. Petrogenic PAH (e.g. fossil fuels) and pyrolytic PAH (e.g. produced by incineration processes) are therefore expected to affect differently organisms. The aim of our study was to measure the effects of pyrolytic and petrogenic PAH on the critical swimming speed (U_{crit}), on the associated energetic costs and on the aerobic metabolic scope in zebrafish *Danio rerio*. Fish were contaminated via trophic pathway with environmentally relevant PAH mixtures at several sub-lethal concentrations. Larvae were fed from the first meal (5 days post fertilization) onward with contaminated, dry and size-adapted pellets. Two-month juveniles and six-month adults were individually challenged following a step protocol in a 170 ml-swim tunnel, where they were forced to swim until fatigue. In parallel, oxygen consumption was measured at each 20 mintue-long step to assess the active and the standard metabolic rate. Aerobic metabolic scope (AMS) was then calculated giving insights into the metabolic performance of D. rerio. While pyrolytic did not show any effect on U_{crit} and AMS, petrogenic contamination had a significant effect on the U_{crit} but not on the associated energetic costs. This suggests that decreasing in locomotor performance was probably due to the morphological deformities observed on the petrogenic contaminated individuals, more than to a limitation in energy supply.

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16:30 Friday 5th July 2013

A11.18

Cardiorespiratory toxicity of commercially-relevant zinc oxide nanoparticles

Tyson J MacCormack (Mount Allison University [MAU], Canada), Kathryn M Butler (MAU, Canada), Alexandra M Blay (MAU, Canada), Neal I Callaghan (MAU, Canada) and Christopher A Dieni (MAU, Canada)

Mammals exhibit a stereotyped cardiorespiratory response to air pollution. Inhalation of nanoparticulate contaminants leads to the development of systemic oxidative stress and long-term cardiovascular morbidity. Water breathing animals are now being exposed to detectable levels of engineered nanoparticles but it is not known whether particulate contaminants exert such effects in aqueous systems. To address this question, white sucker, *Catostomus commersonii*, were fitted with electrocardiography electrodes and exposed to commercially-relevant ZnO nanoparticles (~25 nm diameter, 1 mg/L) for 25 hours. Both Na^+/K^+-ATPase activity and markers of oxidative stress (TBARS) were significantly elevated in gill tissue, suggesting that ZnO nanoparticles compromise the integrity of the epithelium. Liver tissue also exhibited a substantial oxidative stress response, indicating that toxicity was not localized to the gill. Nanoparticle exposure elicited a significant bradycardia after 15 hours, suggesting the particles either stimulated gill oxygen chemoreceptors or that gill damage led to hypoxaemia. The results illustrate that nanoparticles may cause cardiorespiratory toxicity in fish. The cardiorespiratory response of fish to aquatic nanoparticle exposure appears similar to that of mammals exposed to airborne nanoparticle pollution and may serve as a valuable model for *in vivo* nanotoxicity studies.

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16:40 Friday 5th July 2013

A11.19

Impacts of polycyclic aromatic hydrocarbons from the Gulf of Mexico's Deep Water Horizon oil spill on swim performance and aerobic scope of the high performing pelagic mahi-mahi

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During April–July 2010, the largest oceanic oil spill in US history contaminated the Gulf of Mexico with high amounts of polycyclic aromatic hydrocarbons (PAHs) in areas that coincide with the spawning habitats for a number of commercially and ecologically important pelagic top predators. Early life stages of teleost fish are highly sensitive to tricyclic PAHs which lead to cardiac developmental defects. Forty-eight hours of exposure to low levels of PAHs during embryonic development resulted in pericardial oedema, altered atrial-ventricular angle and reduced heart rate in larval mahi-mahi. Following exposure, larval Mahi-mahi were transferred to clear water and raised to 30–40 mm of length (~30 days of age). Swim performance tests and oxygen consumption measurements revealed significantly reduced critical swim speed (U_{crit}), a trend for reduced aerobic scope as well as an apparent increased cost of transport (COT). A similar effect was observed in 30-day-old mahi-mahi exposed to a higher concentration acutely for 24 hours immediately prior to swim performance testing. While reduced cardiac output likely accounts for part of the PAH induced impacts on U_{crit}, neither basal metabolic rate (BMR), maximal metabolic rate (MMR) nor aerobic scope were significantly altered. COT was decreased in exposed fish suggesting impairment of other parameters of importance for swim performance. BMR and MMR were found to scale with size in juvenile Mahi-mahi with exponents of 0.68 and 0.88, respectively illustrating an increased aerobic scope with increased body mass.

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16:50 Friday 5th July 2013

A11.20

Cellular mechanisms for acid–base regulation in elasmobranch gills: VHA translocation in post-feeding sharks and in isolated cells

Jinae Roa (University of California San Diego, United States) and Martin Tresguerres (University of California San Diego, United States)

The gills of dogfish and Atlantic stingray have separate Na^+/K^+-ATPase (NKA)- and V-H^+-ATPase (VHA)-rich cells, which are acid- and base-secreting, respectively. We confirmed that this pattern also applies to leopard shark, mako shark and round stingray, suggesting it is universal for elasmobranches.
Immunostaining of leopard shark (*Triakis semifasciata*) gill sections revealed that NKA- and VHA-rich cells are both mitochondrion-rich (MR). Similar to dogfish, post-feeding alkalosis induced the translocation of VHA from cytoplasmic vesicles to the basolateral membrane of gill cells. To further characterize this mechanism, we isolated and cultured gill cells, some of which stained for cytoplasmic VHA and mitotracker. Addition of cell-permeable cAMP to isolated cells induced the translocation of VHA.
These results suggest that all elasmobranches have similar cellular mechanisms to regulate blood pH, confirm VHA translocation is essential for compensating blood alkalosis, and provide novel insights on the signalling pathway involved.
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10:30 Saturday 6th July 2013

A11.21

Dogfish sharks in low salinity: Rates of acclimation (or lack thereof) in homeostatic variables

Samuel C Guffey (University of Alberta, Canada) and Greg G Goss (University of Alberta, Canada)

Nominally stenohaline, the Pacific dogfish shark (*Squalus suckleyi*) is known to survive excursions into estuaries. To examine their adaptability with respect to various physiological variables, we acutely exposed dogfish to 60% SW and collected blood and water samples over the next 48 hours. We then quantified temporal trends in blood pH, plasma osmolality, and ion concentrations, as well as O_2 consumption and rates of urea efflux.
The rate of O_2 consumption increased around six hours and peaked after 12 hours at 58% above the initial rate before returning to control values. After nine hours, plasma [Cl^-] stabilized at 9% below initial levels, while plasma [Na^+] decreased more than 20% but became relatively stable after 12 hours. Plasma [urea] dropped sharply by 15% between four and six hours and continued to decrease for the entire 48 hours experiment. Surprisingly, the rate of urea efflux increased over time, peaking after 36 hours at 73% above the initial rate. We saw no indication that urea homeostasis would be achieved under these conditions.
Apparently, dogfish in low salinity can maintain homeostasis with respect to some physiological variables, but not others.

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10:40 Saturday 6th July 2013

A11.22

How to get into bones: Proton pump and carbonic anhydrase in *Osedax* boneworms

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Osedax are gutless siboglinid worms that thrive on vertebrate bones lying on the ocean floor, mainly those of whales. The posterior body of female *Osedax* penetrates into bone forming extensions known as 'roots', which host heterotrophic symbiotic bacteria in bacteriocytes beneath the epidermis. The *Osedax* root epithelium presumably absorbs bone collagen and lipids, which are metabolized by the symbiotic bacteria that in turn serve for *Osedax* nutrition. Here we show that *Osedax* roots express extremely high amounts of vacuolar-H^+-ATPase (VHA), which is located in the apical membrane and in cytoplasmic vesicles of root and ovisac epithelial cells.
The enzyme carbonic anhydrase (CA), which catalyses the hydration of CO_2 into H^+ and HCO_3^-, is expressed in roots and throughout *Osedax* body. This suggests that *Osedax* roots have massive acid-secreting capacity via VHA, fuelled by H^+ derived from the CA-catalysed hydration of CO_2 produced by aerobic metabolism. Preliminary transcriptomic analyses revealed mRNAs for several collagenases and nutrient transporter proteins in *Osedax* roots. We propose the secreted acid dissolves the bone carbonate matrix to then allow the absorption of bone-derived nutrients across the skin. We also detected VHA and Na^+/K^+-ATPase (NKA) in gill cells, and NKA in cells of the upper trunk, suggesting a role in HCO_3^- secretion to seawater.
In an exciting example of convergent evolution, this model for acid secretion is remarkably similar to mammalian osteoclast cells. However, while osteoclasts dissolve bone for repairing and remodelling, the *Osedax* root epithelium secretes acid to dissolve foreign bone to access nutrients.

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10:50 Saturday 6th July 2013

A11.23

Differential partitioning of acid and base recovery in the Pacific hagfish (*Eptatretus stoutii*)

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Hagfish (*Eptatretus stoutii*) are thought to possess only a single type of mitochondrial-rich (MR) cell in their gills (cells associated with acid/base and ion regulation). Despite the presence of a single cell type, hagfish are able to readily recover from both acidification and alkalization of the blood. Given the presence of the single MR cell type we hypothesized that hagfish may only be capable of either acid or base recovery through the gill and use a different mechanism to recover the converse stress.
Recent evidence has demonstrated that hagfish are capable of nutrient transport through branchial and extrabranchial mechanisms thus we investigated the role of the skin in ion and acid-base regulation. We also investigated the role of the kidney/gut in acid–base recovery. Using Van-Damm style chambers, we partitioned the flux of ions and net acid from the branchial region separate to the flux from the posterior skin and gut/cloaca.
We found that Hagfish under normal conditions excrete base at a rate of approximately 900 µmol/kg/h through combined posterior skin/cloacal efflux. When infused with acid, hagfish would purge acid loads branchially and reduce base efflux in the posterior region. During alkalosis, the gills do not appear to play a direct role in recovery and net base excretion likely occurs through the gut. We also provide physiological evidence that hagfish excrete ammonia through their skin at rates of approximately 20% of that of the gills.

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11:00 Saturday 6th July 2013

A11.24

Differential mechanisms of Na^+ uptake in freshwater by two populations of pupfish (*Cyprinodon variegatus*)

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The function of Na^+/H^+ exchangers (NHEs) in the apical membrane of fish gills in low Na^+ freshwater is thermodynamically constrained. To compensate, several fishes utilize a NHE-Rh metabolon or carbonic anhydrase (CA) to alter H^+ gradients in micro-domains allowing NHE function despite unfavourable gradients in bulk solutions. The euryhaline pupfish, *Cyprinodon variegatus variegatus* (*Cvv*), can ionoregulate in 2 mM Na+ freshwater and a freshwater population (*C.v. hubbs*i; *Cvh*) can ionoregulate in 0.1 mM Na^+ freshwater. Both populations rely on NHE in dilute freshwater. We comparatively investigated whether an NHE-Rh metabolon or CA facilitates NHE function by simultaneously measuring Na^+ uptake and ammonia excretion while selectively manipulating Na^+, H^+, and ammonia gradients. Fish were also acutely transferred from high to low Na^+ freshwater to monitor transcriptional changes for key genes and CA activity. None of the experiments supported a NHE-Rh metabolon in either population. In contrast, CA appears to play important, but different, roles in Na^+ uptake in the two populations. In *Cvv*, CA activity was comparable in fish acclimated to 7 and 2 mM Na^+, but exposure to ethoxzolamide stimulated Na^+ uptake by 91% and reduced Na^+ uptake by 58%, respectively. In *Cvh*, at 0.1 mM N^+, CA activity increased 75% compared to 7 mM Na^+ fish and ethoxzolamide inhibited Na^+ uptake by 31%. CAc mRNA expression was down-regulated upon transfer to dilute freshwater in both populations, inconsistent with ethoxzolamide and CA activity results. Consequently, we cannot rule out additional unknown mechanisms may also contribute to NHE function.

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11:10 Saturday 6th July 2013

A11.25

Guanylin-regulated transport in the intestinal epithelia of the Gulf toadfish (*Opsanus beta*)

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The homologous peptides, guanylin (GN) and uroguanylin (UGN), are upstream regulators of the cystic fibrosis transmembrane conductance regulator (CFTR) channel, which, in mammals, allows Cl^- secretion and osmotic water movement into the lumen of the intestine. In mammals, these peptides also increase bicarbonate secretion into the lumen, which helps maintain intestinal pH. In the teleost fish intestine, the physiological effects of GN and UGN on water and electrolyte transport have yet to be thoroughly studied. In the present study, the effect of GN, UGN, and renoguanylin (RGN; a GN and UGN homologue) on short-circuit current (ISC) and the transport of bicarbonate and water in the Gulf toadfish (*Opsanus beta*) intestine was determined using Ussing chambers, a pH-stat titration system, and gut sac experiments. GN, UGN, and RGN reversed the ISC of the posterior intestine (absorptive-to-secretory), but not of the anterior intestine. In contrast to mammals, RGN decreased baseline bicarbonate secretion, but, similar to mammals, increased water secretion into the lumen of the posterior intestine. The secretory response of the posterior intestine coincides with the presence of basolateral NKCC and apical CFTR staining of this tissue. Yet, this response to RGN is counterintuitive given the known role of the marine teleost intestine as a compensatory, water-absorbing organ. These data suggest that marine teleosts may possess a tissue-specific secretory response that could be useful for flushing the intestines of calcium carbonate precipitates (formed naturally in the intestines as part of osmoregulatory processes) or other particles

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11:20 Saturday 6th July 2013

A11.26

Evolution of cGMP second messenger signalling systems in vertebrates

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Vertebrate vascular regulation arises from a complex interplay between neural and humoral factors that result in the maintenance of metabolic control through modulatory effects on blood flow. Two significant paracrine factors are the natriuretic peptides (NPs) and the gasotransmitters, most notably nitric oxide (NO). In mammals, vasodilation, and a consequent increase in blood flow, is the predominant effect of all these factors. NO and NPs trigger receptor-mediated signalling cascades that involve production of the second messenger cyclic guanine monophosphate (cGMP), which activates protein kinase G (PKG) resulting in vasodilation. In addition, both NO and NPs are significant vasodilators in amphibians, reptiles and birds. In contrast, we have found that NO and NPs have heterogeneous effects in teleost, cartilaginous and agnathan fishes. In the eel (*Anguilla australis*) both NO and NPs are vasodilators but in the pufferfish (*Contusus brevicaudus*) NO has no effect, although NPs are marked vasodilators. Interestingly, the blood vessels of both species express soluble guanylyl cyclase (sGC) and natriuretic peptide receptor A (NPRA), which are the receptors for NO and NPs, respectively. In the phylogenetically ancient craniate, the hagfish (*Eptatretus cirrhatus*), we found dichotomous results; a vasodilation in response to hagfish NP and a vasoconstriction in response to NO, which raises the question as to whether both molecules signal via cGMP. We will address this by examining cGMP generation and receptor mRNA expression in hagfish. These findings challenge the idea that both NO and NP signalling systems evolved by acting via the cGMP second messenger system.

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11:30 Saturday 6th July 2013

A11.27

Protein expression varies with duration of emersion stress in the salt marsh mussel *Geukensia demissa*

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Most sessile intertidal invertebrates experience varying lengths of emersion (aerial exposure), and therefore hypoxia, at different times in the tidal cycle. The amount and types of cellular stress caused by hypoxia will differ as exposure time increases, likely leading to altered metabolic responses. We examined proteomic responses to increasing emersion times and decreasing recovery (immersion) times in the mussel *Geukensia demissa*, which occurs in salt marshes along the east coast of North America. Individuals are found above mean tide level, and can be emersed for over 12 hours during spring tides. We acclimated mussels to full immersion at 15°C for four weeks, and used proteomic techniques to compare changes in gill protein expression between groups of mussels that continually were immersed (control), were emersed for six hours and immersed during recovery for 18 hours (short), were emersed for 12 hours and recovered for 12 hours (intermediate), or were emersed for 18 hours with a six hour recovery (long). We found clear differences in protein expression patterns among the treatments. Proteins associated with energy metabolism and fermentative pathways increased in abundance after short and intermediate emersion, but not after long-term emersion. Increases in oxidative stress proteins were most apparent after intermediate exposure, and after long-term emersion changes in cytoskeletal protein expression were most notable. We conclude that *G. demissa* alters its strategy for coping with emersion stress depending on the duration of that stress and the amount of time available for recovery.

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11:40 Saturday 6th July 2013

A11.28

RNA-seq reveals a robust transcriptomic response during anoxia in the Western painted turtle

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Western painted turtles, *Chrysemys picta bellii*, can survive four months of anoxia at 3°C and for more than 30 hours at 20°C. Although the physiological responses to anoxia have been well studied, the underlying transcriptomic responses are not as well-characterized. Using RNA-seq methodology, we show a robust transcriptional response in ventricle and telencephalon to 24 hours of anoxia at 19°C, including the up-regulation of several genes by more than 100-fold, despite decreases in total RNA content of 50% and 12% in ventricle and telencephalon, respectively. Quantitative PCR validated the increased expression of several genes in ventricle, including FOS (91-fold) and a GLUT1-like transporter belonging to SLC2A (29-fold). Similar validations were made in telencephalon for APOLD1 (292-fold) and PTGS2 (219-fold). There were no decreases in telencephalic gene expression, but there were decreases in five ventricular genes, including *CDO1*, which, when knocked out in mice, increases hydrogen sulphide levels, *SRSF5*, a gene involved in mRNA processing, and *MKNK1*, which regulates growth and differentiation.
This study shows how the increased availability of sequenced genomes can provide an unprecedented level of insight into our understanding of extreme physiological adaptation and marks a transformative moment in comparative physiology.

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11:50 Saturday 6th July 2013

A11.29

Massive increase of NO metabolites in red muscle of anoxic crucian carp

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We report here an anoxia induced increase in the NO metabolites nitrite, S-nitrosylated (SNO) and iron-nitrosylated species in red muscle of crucian carp (*Carassius carassius*). This fresh water fish is extremely anoxia-tolerant, being able to survive for several months completely without oxygen. The crucian carp is therefore a perfect model for studying how damage from anoxia and reoxygenation can be avoided.
S-nitrosylation of mitochondrial proteins has been shown to protect mammalian tissues against ischemia/reperfusion damage. Nitrite can act as a source for nitric oxide (NO) in hypoxic conditions, and can cause S-nitrosylation of proteins. Previously, we have shown that NO metabolites are accumulating up to 10-fold in the hearts of anoxic crucian carps and we believe that this may protect the heart from reoxygenation damage.
The heart in crucian carp is fully active in anoxia, and thus may need particular protection. Other tissues had either maintained or slightly increased NO metabolite levels. We now report that NO metabolite levels in red muscle also increase massively during anoxia, which may reflect that the high density of mitochondria in this tissue needs protection against reoxygenation damage. The red muscle is also an active tissue in anoxia, because it is the site for conversion of lactate to ethanol – the main anaerobic end product in crucian carp. We are now searching for common pathways for protection in the heart and red muscle.

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12:00 Saturday 6th July 2013

A11.30

Metabolic fate of lactate after anoxia at 20°C in the Western painted turtle

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Western painted turtles (*Chrysemys picta bellii*) can survive anoxia for 120 days at 3°C and tolerate plasma lactate concentrations approaching 200 mM. The metabolic fate of lactate accumulated over six hours of anoxia and processed over 39 hours of recovery at 20°C was investigated with stable isotope tracer methodology. A bolus infusion of [U-^{13}C]-lactate tracer was given via an intra-arterial catheter two hours into anoxia. ^{13}C enrichment of metabolites and CO_2 from blood, bone, and expired air were measured using gas chromatography and isotope ratio mass spectrometry. The tracer completely equilibrated with the lactate pool by the end of anoxia. M+3-glucose enrichment slowly increased throughout the recovery period, and liver glycogen accounted for ~30% of the label at the end of recovery. CO_2 was the fate for ~26% of the lactate carbon, and CO_2 exchange with the bone accounted for ~17% of the label, indicating that the shell is an important sink for metabolically produced CO_2. The plasma concentration and enrichment of beta-hydroxybutyrate also increased. Our results demonstrate that painted turtles oxidize part of the lactate load initially during recovery from anoxia, but that the primary fate is gluconeogenesis.
This research was funded by Saint Louis University and the National Institutes of Health.

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12:10 Saturday 6th July 2013

A11.31

Light pollution at night advances reproductive function in a nocturnal primate

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Light pollution altering light/dark cycles constitutes a new threat for biodiversity. However, despite many observations on the effects of artificial lighting on fauna, few experimental studies have documented its consequences on nocturnal mammals especially in terms of reproduction. The impact of light pollution was studied on a nocturnal primate, the grey mouse lemur (*Microcebus murinus*), of which the breeding season is strongly dependent on the photoperiod. In this primate, females remain in an inactive sexual state during short-photoperiod and enter oestrus at the beginning of long-photoperiod. During five weeks, at the transition between short-photoperiod (SD: 10/14) and long-photoperiod (LD: 14/10), six adult females were exposed to light pollution at night (yellow LED) and compared to six females non-exposed to light pollution. Reproductive state was monitored by morphological changes in the vulva (reddening, swelling and perforation) and by urinary 17- β-estradiol levels. In addition, daily rhythms of locomotor activity and core temperature were recorded by telemetry. Oestrus occurrences and peaks of urinary estradiol were significantly advanced (by 10 days) in females exposed to light pollution compared to control females. Moreover, daily rhythms were modified under light pollution as exemplified by locomotor activity phase delay and increases in core temperature. This study evidenced that light pollution affects reproductive physiology and daily rhythms in a nocturnal primate with potential consequences in terms of maladaptive responses to seasonal environmental changes.

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13:20 Saturday 6th July 2013

A11.32

Sex, colour and stress: Unravelling the role of stress and reproductive hormones in rapid colour change in an amphibian

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Some animals have the capacity to change their colour, sometimes dramatically but how does this animal change colour; and why does it change colour? Understanding how colour change occurs will often help determine why. Here we demonstrate dynamic colour change in an anuran and investigate its regulatory mechanisms. *Litoria wilcoxii* rapidly changes from brown to yellow during amplexus; we show this by comparing dorsal colour of unpaired and amplecting males. Colour change involves the movement of pigments in chromatophores activated by hormones. We investigated whether corticotropin (ACTH) and neurohormone (epinephrine) or male reproductive hormone (testosterone) were triggering colour change. Males were injected with epinephrine (n=5), ACTH (n=6), and testosterone (n=5), as well as saline (n=5) and sesame oil (n-5) as controls. Colour was measured using digital photography. Epinephrine-injected frogs underwent a significant colour change to bright yellow within 10 minutes compared to ACTH and control frogs which did not change colour. Testosterone-treated males underwent a much slower (1 hour) and less yellow change. These results suggest that even though the hypothalamo–pituitary gonadal (HPG) axis is important for the expression of sexual morphological traits in male frogs, it is the stress-axis, particularly neurotransmitters, that mediates the link between physiological and morphological traits (rapid colour change).The results have opened up opportunities for future research to unravel the functions of physiological systems in amphibian colour change in breeding.

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13:30 Saturday 6th July 2013

A11.33

The evocative scent of male urine: Identification of the tilapia sex pheromone

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In many terrestrial and freshwater vertebrates chemical communication is mediated by pheromones carried in urine. Male Mozambique tilapia (*Oreochromis mossambicus*) signal dominance by increasing urination frequency during territorial disputes and courtship; the olfactory potency of the urine depends on their social rank. Females spawn preferentially with dominant males and exposure to male urine increases 17α,20β-dihydroxypregn-4-en-3-one (17,20β-P) metabolism (a steroid known for stimulating oocyte maturation in fish). The chemical identity of the compounds involved is unknown and their pheromonal action(s) not fully understood. The aims of this study were to identify the sex pheromone in dominant male urine and assess its physiological effect(s) on females. First, we combined HPLC and electro-olfactogram recording to target the most potent urinary fraction. Using mass spectrometry and NMR-spectroscopy, we identified two compounds as isomeric steroids (pregnanetriol-3-glucuronides). We synthesized both steroids and verified their olfactory potency; both sexes produced strong sigmoidal concentration-response curves. Second, we exposed females to the synthetic steroids and measured subsequent 17,20β-P release. We observed a rapid 15-fold increase in 17,20β-P release after exposure to the steroids or male urine, but not the aqueous urine fraction. This suggests males release these steroids to stimulate final oocyte maturation in females. In conclusion, tilapia urine contains a sex pheromone that by influencing female endocrinology, promotes spawning synchrony.

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13:40 Saturday 6th July 2013

A11.34

Dietary astaxanthin: Impacts on mate-choice in cherry barbs and maternal carotenoid transfer

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The objectives of this study were to determine:
(i) the effects of a novel, naturally sourced astaxanthin on adult cherry barb (*Puntius titteya*) colour morphology;
(ii) the impacts of any colour change on male mate choice; and
(iii) whether maternal astaxanthin consumption affected offspring development.
Astaxanthin is a carotenoid pigment with important antioxidant properties that most animals, including fish, are incapable of synthesizing and, therefore, must obtain through the diet. Dietary carotenoids have the potential to alter fish colouration and in nature can confer information regarding health and/or sexual fitness between conspecifics. Within the ornamental fish industry, dietary carotenoids present the opportunity to enhance fish colouration and increase their commercial value so diets containing novel carotenoid sources are routinely screened. However, the wider impacts of subsequent dietary induced colour changes, such as those to colour-based behaviours or trans-generational effects, are rarely considered.
Here, groups of adult male and female barbs were fed either a control diet (no astaxanthin), a synthetic astaxanthin (positive control) or a novel carotenoid diet (at 50 ppm or 100 ppm). Changes in colouration were measured over a 12-week period via digital SLR photography. Male choice of females fed these diets was assessed and offspring from mothers fed these diets were assessed for growth, metabolic rate, carotenoid content and susceptibility to UV damage. The novel carotenoid diet was found to alter colouration; subsequent effects on mate choice and offspring quality will be discussed.

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13:50 Saturday 6th July 2013

A11.35

Characteristics of cardiovascular regulatory development in birds: Inferences for the ancestral features of this process in non-passerine birds

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Details of cardiovascular development in birds have fascinated scientist since the age of Socrates and Aristotle. The classic model of 'bird' development, the embryonic chicken (*Gallus domesticus*) has been valuable in identifying key developmental events as well as documenting developmental plasticity of cardiovascular function.
Given the diversity of birds and the morphological and neonatal maturity differences, features of cardiovascular development in chicken strains may not apply universally to avian embryos. Further study of single species makes speculation regarding the evolution of cardiovascular development in birds impossible.
Recent studies on multiple precocial avian species have begun to illustrate commonalities shared by species during embryonic development. In general, arterial pressure rises continuously while heart rate remains relatively stable during the final 50% of embryogenesis in these precocial birds. Species studied to date exhibit similar pronounced adrenergic receptor tone on the cardiovascular system. Cholinergic receptor tone has been identified in all species studied with the exception of specific chicken breeds.
Based on these findings we hypothesize that the embryonic maturation and presence of these two cardiovascular regulatory elements represents the ansectrial condition for non-passerine bird species.

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14:00 Saturday 6th July 2013

A11.36

A postprandial oxygen supply–demand mismatch triggers cardiac hypertrophy: Results from comparative studies of pythons and gators

Christopher E Slay (University of California Irvine, United States), Sanne Enok (Aarhus University, Denmark), Tobias Wang (Aarhus University, Denmark) and James W Hicks (University of California Irvine, United States)

Cardiac hypertrophy (enlargement of the heart) is known to occur in response to both reduced oxygen supply and increased oxygen demand in lower vertebrates. The Burmese python (*Python molurus*) has served as a model of cardiovascular response to one such period of elevated aerobic demand, digestion, and is notable for its capability to rapidly and massively remodel the heart, presumably to facilitate oxygen transport. This postprandial cardiac remodelling is a facultative rather than obligatory response, and in this series of studies we sought to determine the 'trigger' for postprandial cardiac hypertrophy. We predicted hypertrophy occurs when oxygen demand exceeds oxygen delivery capacity, and utilizing an experimental approach whereby oxygen supply is reduced by ~50% (via rendering animals anaemic) we investigated the cardiovascular response to digestion in both Burmese pythons and American alligators (*Alligator mississippiensis*). Pythons rendered anaemic and fed rodent meals equivalent to 25% of body mass experienced a 125% increase in heart rate and 38% larger ventricles compared to fasted normoxic controls. Alligators rendered anaemic and fed *ad libitum* ate meals energetically equivalent to rodent meals of 5.6% of body mass, and experienced a more modest 74% increase in heart rate and no increase in ventricular mass over fasted controls. We argue that the capacity of the cardiovascular system to meet the demand for convective oxygen transport serves as the primary determinant of whether postprandial cardiac hypertrophy occurs.

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14:10 Saturday 6th July 2013

A11.37

Introduction of the stress hormone cortisol through food pellets induces cardiac remodelling in rainbow trout *Oncorhynchus mykiss*

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Factors compromising fishes' ability to handle acute stressors are largely unknown. Salmonids consistently responding to stress with high plasma levels of the stress hormone cortisol have bigger hearts than fish responding with low cortisol. mRNA levels of molecular markers of cardiac hypertrophy and disease, used in mammalian cardiology, were up-regulated in the bigger hearts and focal fibrosis indicated cardiac pathology. In the present study, we investigated whether cortisol, introduced in the feed, could directly induce cardiac remodelling. Forty-five days of cortisol treatment induced a 34% increase in cardiosomatic index (CSI; ventricular weight/body weight), compared to controls (p<0.0001, n=36, 34). A consistently higher CSI in the cortisol-treated groups was found for 70 (53%, p<0.0001, n=14, 3), 80 (49%, p<0.001, n=10, 3) and 90 days (40%, p<0.0001, n=15) of cortisol treatment. Of notice, ventricular weight was increased despite of general body weight loss. Molecular markers of pathological hypertrophy and heart failure, i.e. SMLC2 and VMHC mRNA and ANP and VNP mRNA, respectively, were up-regulated following 45 and 90 days of cortisol treatment. During physiological adaptation, increased heart size is coupled to increased swimming performance. In contrast, a significantly reduced swimming performance, assessed by swimming respirometry, in cortisol-treated fish (p<0.05, n=10, 6) suggested that cortisol-induced heart growth in salmonids is not adaptive of nature.

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14:20 Saturday 6th July 2013

A11.38

Spatial variation in the relationship between performance and metabolic rate in wild juvenile Atlantic salmon

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Maintenance metabolic rate (MR, the energy cost of self-maintenance) is linked to behavioural traits and fitness and varies substantially within populations. Despite having received much attention, the causes and consequences of this variation remain obscure. Theoretically, such within-population variation in fitness-related traits can be maintained by environmental heterogeneity in selection patterns, but for MR this has rarely been tested in nature. Here, we experimentally test if the relationship between MR and performance can vary spatially by assessing survival, growth rate and movement of Atlantic salmon (*Salmo salar* L.) juveniles from 10 family groups differing in MR (measured as egg metabolism) that were stocked in parallel across 10 tributaries of a single watershed. The relationship between MR and relative survival and growth rate varied significantly among tributaries. Specifically, the effect of MR ranged from negative to positive for relative survival, whereas it was negative for growth rate. The association between MR and movement was positive and did not vary significantly among tributaries. These results are consistent with a fitness cost of traits associated with behavioural dominance that varies across relatively small spatial scales (within a single watershed). More generally our results support the hypothesis that spatial heterogeneity in environmental conditions contributes to maintain within-population variation in fitness-related traits, such as MR.

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14:30 Saturday 6th July 2013

A11.39

Environmental stress in sea urchin: Involvement of nitric oxide

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In invertebrates, the pluripotent gas, nitric oxide (NO) is involved in a variety of processes including development, feeding, defence, bioluminescence, neural transmission, immune response, swimming. In sea urchin, NO is implicated in important processes occurring at fertilization and at later developmental stages, where it acts as a signalling molecule during settlement and metamorphosis. Recently, it has been reported that NO is a cellular signal of environmental stress. In fact, NO production is the first response of *Paracentrotus lividusse*a urchin embryos to the diatom-derived aldehyde decadienal and that this gas mediates the response to this bioactive agent (Romano et al., 2011). In this context, our focus is to investigate if this messenger plays a role as a universal sensor of different environmental stress agents in the sea urchin embryo or if embryos display different responses following different treatments. To this aim, we used cadmium and manganese, well noted metals that induce developmental delay and abnormalities in sea urchin embryos, mainly in relation to skeleton elongation. To understand the involvement of NO in these processes, embryos were treated with different metal concentrations under reduced endogenous NO levels, using the NOS inhibitor TRIM. An increase in the number of abnormal plutei at increasing TRIM concentrations was observed, suggesting a protective role of this messenger in the stress response induced by these agents. Moreover, NO signalling in treated embryos was examined by following the formation of nitrated proteins and also the expression of some stress and skeletogenic genes was revealed by real time q-PCR.

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14:40 Saturday 6th July 2013

A11.40

Benthic hypoxia and anoxia: A major blow for biodiversity and ecosystem processes in shallow coastal seas

Bettina Riedel (University of Vienna, Austria) and Michael Stachowitsch (University of Vienna, Austria)

The deterioration of the marine environment has put us into a new era of marine research devoted to studying degraded habitats. While microorganisms may 'rule the Earth', it is the macrofauna that fundamentally defines ecosystems and their functioning. With more than 500 identified dead zones, coastal hypoxia and anoxia have grown into a global threat to marine biodiversity. Often the onset and extent of oxygen depletion events are difficult to predict, hindering full documentation in the field. By integrating both physiological function and ecological processes, behavioural parameters are ideal for assessing the stress status of benthic macrofauna to low dissolved oxygen. The initial response of individuals to hypoxia can serve as an early-warning signal of environmental change, while the subsequent change in behavioural reactions of species during ongoing perturbation indicates the level of hypoxia and helps assess community degradation. Using a benthic chamber equipped with a time-lapse camera and microsensors, we induced small-scale anoxia in a benthic soft-bottom community setting in 24 m depth in the Northern Adriatic (Mediterranean). Oxygen depletion elicited significant and predictable changes in behavioural reactions in virtually all organisms; most atypical behaviours were associated with specific oxygen thresholds. The results also revealed changes in intra- and interspecific reactions (i.e. shelter seeking, defensive and territorial behaviour, predator–prey relationship) and sequences in tolerance/mortality. The integrated approach complements laboratory studies and helps transport complex ecological processes and responses to the public and decision-makers for developing specific monitoring, assessment and conservation plans.

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14:50 Saturday 6th July 2013

A11.41

Evaluating marine mammal behaviour to mitigate depredation within an ecosystem-based approach to fisheries management

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Killer whales (*Orcinus orca*) are globally distributed and well-known for their varied and innovative hunting strategies. They frequently interact with longline fishing operations around the world and depredation of commercial catches can lead to substantial reductions in yield. Killer whales have frequently been observed to interact with longline fisheries for toothfish (*Dissostichus eleginoides*) in the Indian Ocean, particularly around the Kerguelen Islands, Prince Edward and Marion Islands and in the South Atlantic around South Georgia. Anecdotal accounts from fishermen attribute significant losses of catch to this activity. Numerous mitigation measures have been trialled at South Georgia though none with any real success and the problem of depredation persists. In this study we characterize the seasonal and spatial distribution and behavioural patterns of killer whales around South Georgia, using marine mammal sighting data from on-board fisheries observers for the period 1996 to 2012. Killer whales show seasonal movement patterns that coincide with the seasonal presence and abundance of key prey species, specifically Antarctic fur seals (*Arctocephalus gazella*), although interactions with fishing vessels may modify this behaviour. The impact and interaction of higher predators with fisheries is an important consideration within an ecosystem approach to sustainable fisheries management. We review the success of mitigation measures to date and consider how an improved understanding of higher predator behaviour can be used to inform fisheries management decisions.

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15:00 Saturday 6th July 2013

A11.42

Physiological vagility explains genetic isolation by distance in vertebrates

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Previous studies analysing population differentiation of neutral microsatellite markers generally use an array of physical environmental characteristics to explain differences in genetic exchange. We introduce a quantitative definition for vagility that incorporates aerobic capacity, body size, body temperature, and the metabolic cost of transport. We test whether this definition correlates with empirical data for maximal dispersal distances and measured microsatellite genetic differentiation with distance for vertebrates.

Both log maximal dispersal distance and log vagility increase and scale in a similar manner with log body mass, indicating that vagility is a good predictor of maximal dispersal distances. Genetic differentiation with distance was greater for terrestrial locomotion compared to flight and swimming, with amphibians showing the greatest mean and variance in differentiation. Flying birds and mammals and swimming marine mammals showed the least genetic differentiation with distance indicative of the lower cost of transport. Genetic differentiation with distance was not related to body mass in any class or with any mode of locomotion, indicating that the results are explained by a combination of aerobic capacity and metabolic cost of transport.

These results demonstrate that interspecific differences in physiological vagility explain genetic isolation by distance in tetrapod vertebrates.

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15:40 Saturday 6th July 2013

A11.43

Comparative reptile transcriptomics reveals the true complexity of snake venom

John F Mulley (Bangor University, United Kingdom), Adam D Hargreaves (Bangor University, United Kingdom), Martin T Swain (Aberystwyth University, United Kingdom) and Darren Logan (Wellcome Trust Sanger Institute, United Kingdom)

Snake venom is frequently cited as being highly complex or diverse and a large number of venom toxin genes and gene families have been identified, predominantly from EST-based studies of venom glands during the re-synthesis of venom following manually-induced emptying. It has been suggested that many of these gene families have originated via the duplication of a gene encoding a non-venom protein expressed elsewhere in the body followed by recruitment into the venom gland where natural selection can act to increase toxicity, with subsequent additional duplications leading to a diversification within gene families.

The apparent widespread distribution of genes known to encode venom toxins in snakes in the salivary glands of a diverse set of reptiles led to the development of the Toxicofera hypothesis – the single, early evolution of venom in reptiles. However, the identification of many of these genes as venom components is based largely on their expression in the venom gland during venom synthesis.

We have carried out a comparative transcriptomic survey of three tissues in five reptile species and have found that many of the genes though to encode venom toxins are in fact expressed in multiple tissues and therefore represent 'housekeeping' or maintenance genes. The complexity and diversity of snake venom has therefore been over-estimated and many of the genes used to support the single early evolution in reptiles are in fact not venom at all.

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15:50 Saturday 6th July 2013

A11.44

Long antibody persistence and transgenerational immunoresponse in a long-lived vertebrate with potential implications for conservation of Procellariiform seabirds

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Animals are expected to trade-off the energy invested into various components of their immune system across their life spans to optimize their fitness. In birds, one component of this trade-off is the persistence of immunoglobulins in adults, but also in offspring to which they are transferred via the egg yolk.
Although little studied in natural populations, the persistence of immunoglobulins has the potential to dramatically affect the dynamics of immunity and the outcome of host–pathogen interactions. A key issue is whether antibody level against a specific antigen can persist over several years and whether maternal antibodies against that antigen could persist several weeks in offspring several years after the mother was exposed.
By means of a multiple year vaccination design against Newcastle Disease Virus, we experimentally addressed these questions in females of a long-lived Procellariiform seabird, the Cory's shearwater *Calonectris borealis*. We found that the immunological profiles against specific antibodies were highly repeatable among years, reflecting a high interannual persistence of antibody levels in adult females. We also found a high transfer rate and a long persistence of maternal antibodies in offspring, further suggesting that antibody persistence may be an important trait for the individual protection against pathogens not only in adults but also in offspring.
These results illustrate the need to consider the dynamics of the immune responses at different temporal scales if we are to understand the evolutionary ecology of host–pathogen interactions in such long-lived wild species, but also if are to imagine using vaccination as management tools.

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16:00 Saturday 6th July 2013

A11.45

Is there a link between innate immunity, resting metabolic rate and overwinter survival of the root vole, *Microtus oeconomus*?

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White blood cells (WBCs) count, H/L (granulocytes/lymphocytes) ratio and NAbs (natural antibodies) characterize the first line of innate immunity defence against parasites. It has been hypothesized that acquiring this immunity is metabolically costly and thus, along with other energy expenditures, may affect overwinter survival. To test this we analysed the relationship between WBC, H/L, Nabs and resting metabolic rate (RMR) over two winters in a wild population of the root vole. H/L, but not WBC and Nabs differed significantly between winters. Also, only H/L ratio was positively correlated with RMR. We, thus, suggest that an increase of the pool of circulating granulocytes manifested as an elevated H/L ratio) was reflected in the increase in the metabolic costs of vole's maintenance. We associate it with the prevalence of infection with the blood parasite *Babesia* ssp. concurrently documented in our study population and affecting overwinter survival.

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16:10 Saturday 6th July 2013

A11.46

The rumblings of the Shorthorn sculpin gut! Effects of food on motility in the proximal intestine of a fish species

Jeroen Brijs (University of Gothenburg, Sweden), Grant Hennig (University of Nevada, United States), Michael Axelsson (University of Gothenburg, Sweden) and Catharina Olsson (University of Gothenburg, Sweden)

Gastrointestinal motility is an important part of the digestive process in fish. Alas, relatively little is known about the types of motility patterns and the effects of feeding on these patterns in the gastrointestinal tract in ectotherms. In this study, *in vivo* video recordings and spatiotemporal maps were used to characterize the motility patterns in both fed and fasted Shorthorn sculpin (*Myoxocephalus scorpius*). Spatiotemporal maps were used to portray motility patterns and allowed quantification of a number of motility parameters.
In both fed and fasted fish we observed circular muscle contractions with relatively short durations and small amplitudes that mainly propagated orally at ~0.4–0.6 mm s^{-1}. These contractions are reminiscent of myogenic 'ripples' recorded in mammals. Prolonged, larger amplitude circular muscle contractions, similar to migrating motor complexes in mammals, mainly propagated anally at ~0.2 mm s^{-1}. Fed fish had, on average, half as many prolonged, anally propagating contractions (~7/hour) and twice the number of non-propagating contractions (~12/hour) than fasted fish.
These results show that the presence of food can modulate gastrointestinal motor patterns in fish, to presumably maximize mixing in the fed state by increasing the number and strength of non-propagating contractions. The increased prevalence of prolonged, anally-propagating contractions in fasted fish suggests that they may have a similar role to migrating motor complexes in mammals; their 'sweeping' action may remove food remnants and prevent the establishment of pathogens, thus maintaining a healthy, functional gastrointestinal system in between feedings.

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16:20 Saturday 6th July 2013

A11.47

Among-sibling differences in the phenotypes of juvenile fish depend on their location within the ovary

Tim Burton (University of Glasgow, United Kingdom), Mia O Hoogenboom (James Cook University, Australia), Nicholas D Beevers (University of Glasgow, United Kingdom), John D Armstrong (Marine Scotland, United Kingdom) and Neil B Metcalfe (University of Glasgow, United Kingdom)

We investigated whether among-sibling differences in the phenotypes of juvenile fish were systematically related to the position in the ovary where each individual developed during oogenesis. We sampled eggs from the front, middle and rear thirds of the ovary in female brown trout of known dominance rank. In the resulting juveniles, we then measured traits that are related to individual fitness: body size, social status and standard metabolic rate (SMR).
When controlling for differences among females in mean egg size, siblings from dominant mothers were initially larger (and had a lower mass-corrected SMR) if they developed from eggs at the rear of the ovary. However, heterogeneity in the size of siblings from different positions in the ovary diminished in lower ranking females. Location of the egg within the ovary also affected the social dominance of the resulting juvenile fish, although the direction of this effect varied with developmental age.

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16:30 Saturday 6th July 2013

A11.48

Sleep-like states and discontinuous breathing in the cockroach *Nauphoeta cinerea*

Philip GD Matthews (University of Queensland, Australia)

Discontinuous breathing patterns characterized by alternating periods of breath-holding and ventilation occur throughout the animal kingdom, from vertebrates to invertebrates. Many insects are known to display discontinuous gas exchange cycles (DGCs), but only during periods of inactivity. In *Drosophila*, periodic bouts of inactivity of more than minutes show all the hallmarks of sleep (increased arousal threshold, homeostatic control), presenting the possibility that DGCs arise in insects when they enter quiescent, sleep-like states.

To test for an interaction between the gas exchange pattern adopted by an insect and its level of arousal or 'wakefulness', speckled cockroaches *Nauphoeta cinerea* were placed in respirometry chambers beneath a video tracking system to allow simultaneous measurement of gas exchange pattern and activity. Arousal levels during different breathing patterns were determined by exposing cockroaches to an unpleasant stimulus (either green light 505 nm, or 100 Hz vibration) and recording the time taken to respond. Furthermore, pharmacological agents shown to alter sleep duration in *Drosophila* were fed to cockroaches to determine whether this alters the occurrence of discontinuous breathing.

The results of these experiments will be discussed.

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16:40 Saturday 6th July 2013

A11.49

Nocturnal orientation in a day-active dung beetle

Jochen Smolka (Lund University, Sweden), Emily Baird (Lund University, Sweden), Marcus J Byrne (University of the Witwatersrand, South Africa), Basil El Jundi (Lund University, Sweden), Eric J Warrant (Lund University, Sweden) and Marie Dacke (Lund University, Sweden)

Nocturnal dung beetles remain the only insects shown to use the polarization pattern around the moon and the Milky Way as directional cues for orientation. Supposedly, their highly adapted visual systems – with larger lenses, wider and longer receptors and a tracheal tapetum – enable them to perform this difficult task. We show here that even a diurnal dung beetle, without specializations for dim-light vision, can use the moon, the lunar polarization pattern and the Milky Way to keep a straight course.

We compared the exclusively diurnal *Scarabaeus lamarcki* and the nocturnal *S. satyrus* when rolling their dung-balls in their natural habitat on either:

(1) a moonlit night (moon visible/shaded);

(2) a moonless night; or

(3) with an artificial light.

For each condition, beetles were filmed exiting a 3-m arena. Orientation performance was evaluated from the length of the beetles' outbound paths. While nocturnal beetles performed equally under all conditions, the paths of diurnal animals were about 50% longer when the moon was absent. However, the majority of beetles – diurnal and nocturnal – could clearly use the stars as an orientation cue. Beetles were then tested in the laboratory to determine the minimum intensity of a point light source necessary for orientation. Under these conditions, as under a moonlit sky, we found no difference in orientation performance between diurnal and nocturnal beetles.

We will discuss the implications for our interpretation of visual adaptations to nocturnality, and the potential for nocturnal navigation in a large number of insect species.

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16:50 Saturday 6th July 2013

A11.50

Effect of fermented moist feed by *Lactobacillus plantarum* on *Salmonella typhimurium* sl 1344 nal^r in SPF chicken

Nabil Wali (University of Plymouth, United Kingdom)

Salmonellosis is a zoonotic disease (food born disease). Poultry and poultry products are the main source of diseases in human. This pilot study is to check the effect of moist feed fermented by *Lactobacillus plantarum* on *Salmonella typhimurium* nalr in digestive system of broiler chicks and the effect of probiotic on the microbial population diversity changes in the intestine.

Fourteen chicks were randomly allocated to two treatments. Seven per treatment divided in to two pens. Control (CON): chick's food with no adding *L. plantarum*. The second group is Fermented moist food (FMF): chicken fed moist normal food but fermented by *L. plantarum* NCIMB 41606 at 30°C for 24 hours, from day one of age.

The results indicate the survival of administered *L. plantarum* NCIMB 41606 via the condition of gastrointestinal tract of chicken. Extreme reduction of *S. typhimurium* nalr NCIMB1344 in the digestive system of chicken by moist feed fermented by *L. plantarum* as well as complete colonization of intestine by the bacteria which has administered. Better way to reduce *Salmonella* in chicken is by prevent colonization in the intestine. The changing the microorganism population diversity in the gastrointestinal tract of chicken by using FMF.

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Poster session – Friday 5th July 2013

A11.51

Balancing the competing requirements of air-breathing and display behaviour during male–male interactions in Siamese fighting fish *Betta splendens*

Steven J Portugal (Royal Veterinary College, United Kingdom), Lesley Alton (University of Queensland, Australia) and Craig White (University of Queensland, Australia)

Air-breathing fish of the Anabantoidei group meet their metabolic requirements for oxygen through both aerial and aquatic gas exchange. Siamese fighting fish *Betta splendens* are anabantoids that frequently engage in aggressive male–male interactions which cause significant increases in metabolic rate and oxygen requirements. These interactions involve opercular flaring behaviour that is thought to limit aquatic oxygen uptake, and combines with the increase in metabolic rate to cause an increase in airbreathing behaviour. Airbreathing events interrupt display behaviour and increase risk of predation, raising the question of how Siamese fighting fish manage their oxygen requirements during agonistic encounters.

Using open-flow respirometry, we measured rate of oxygen consumption in displaying fish to determine if males increase oxygen uptake per breath to minimize visits to the surface, or increase their reliance on aquatic oxygen uptake. We found that the increased oxygen requirements of Siamese fighting fish during display behaviour were met by increased oxygen uptake from the air with no significant changes in aquatic oxygen uptake. The increased aerial oxygen uptake was achieved almost entirely by an increase in airbreathing frequency.

We conclude that limitations imposed by the reduced gill surface area of airbreathing fish restrict the ability of Siamese fighting fish to increase aquatic uptake, and limitations of the airbreathing organ of anabantoids largely restrict their capacity to increase oxygen uptake per breath.

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Poster session – Friday 5th July 2013

A11.53

Acid-trapping ammonia excretion by ionocytes of medaka (*Oryzias latipes*) acclimated to seawater

Li-Yih Lin (National Taiwan Normal University, Taiwan)

The acid-trapping mechanism is critical for a fish's ability to excrete ammonia in fresh water. However, it remains unclear whether acid-trapping is also critical for ammonia excretion in seawater (SW).
Using a scanning ion-selective electrode technique (SIET) to measure H^+ gradients, an acidic boundary layer was detected at the yolk-sac surface of SW-acclimated medaka (*Oryzias latipes*) larvae. The H^+ gradient detected at the surface of ionocytes was higher than that of keratinocytes in the yolk-sac. Treatment with tricine buffer or EIPA (a Na^+/H^+ exchanger inhibitor) reduced the H^+ gradient and ammonia excretion of larvae.
In situ hybridization and immunochemistry showed that slc9a2 (NHE2) and slc9a3 (NHE3) were expressed in the same SW-type ionocytes. A real-time PCR analysis showed that transfer to SW down-regulated branchial mRNA expressions of slc9a3 and rhesus glycoproteins (rhcg1, rhcg2, and rhbg) but up-regulated that of slc9a2. However, slc9a3, rhcg1, rhcg2, and rhbg expressions were induced by high ammonia in SW.
This study suggests that ionocytes play a critical role in acid and ammonia excretion, and that the Na^+/H^+ exchanger and Rh glycoproteins are involved in the acid-trapping ammonia excretion mechanism.

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Poster session – Friday 5th July 2013

A11.54

Ontogeny of osmoregulatory organs in three finfish species: Mahi-mahi, cobia and yellowfin tuna

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Mahi-mahi (*Coryphaena hippurus*), Cobia (*Rachycentron canadum*) and yellowfin tuna (*Thunnus alalunga*) are pelagic species of high commercial and recreational importance. However, little attention has been paid to the development of physiological functions in early life stages. Brood stock of these species were captured and maintained at RSMAS, University of Miami (cobia and mahi-mahi) or at the IATTC Achotines Laboratory, Panama (yellowfin tuna). All brood stock spawned spontaneously allowing access to freshly fertilized embryos. The ontogeny of development of osmoregulatory organs, gills, intestine and kidney as well as ontogeny of active osmoregulation, are studied at early life stages, using four different approaches at cellular to integrative scales. Histomorphology staining was used to follow development of osmoregulatory organs and followed by immunocytochemistry and molecular quantification to examine the cellular distribution, and the expression of different proteins such as Na^+/K^+-ATPase (NKA), $Na^+/K^+/2Cl^-$ co-transporter (NKCC), and the chloride channel (CFTR), in ion-transporting epithelia of all three species. These three species are all pelagic and highly migratory with a high growth rate; however their distribution is different. While yellowfin tuna is strictly found in open water, mahi-mahi and cobia are also distributed either near the coast or even in mangrove sloughs, potentially presenting salinity variations. In addition, these species likely represent different osmo-respiratory compromises. These differences may lead to differences in their strategy of development during ontogeny. In this comparative study, ongoing efforts are focused on improving knowledge of the development of osmoregulatory function in pelagic species.

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Poster session – Friday 5th July 2013

A11.55

The ontogeny of blood viscosity and haematocrit in the American alligator, *Alligator mississippiensis*

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Wall shear stress is an important cue for development and regulation of the cardiovascular system and is proportional to blood viscosity. Viscosity is largely determined by haematocrit and is a major contributor to peripheral cardiovascular resistance. The ontogeny of blood viscosity is virtually unknown in non-mammalian vertebrates but is an important variable that influences cardiac work, O_2 transport, cardiac output, and vascular function. We measured blood viscosity and haematocrit in the American alligator, *Alligator mississippiensis*, at two time points during incubation and in animals up to 2.5 m total length. Blood viscosity and haematocrit at 90% incubation were significantly greater than in juveniles and adults, but plasma viscosities were not different. Alligator blood viscosity was highly shear dependent and varied nonlinearly with haematocrit in all groups. Post-hatching blood viscosity and haematocrit increased proportionally with total body length and were similar to values reported for other crocodilians. Therefore, ontogenetic blood viscosity differences in American alligators are primarily the consequence of size specific haematocrit variation.
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Poster session – Friday 5th July 2013

A11.56

Age-dependent role of angiotensin II regulation of cardiovascular function in the American alligator (*Alligator mississippiensis*)

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Angiotensin II (Ang II) is an essential regulator of blood volume and blood pressure. During bouts of hypovolaemia or hypotension stress, Ang II is critical for the reestablishment of normotensive arterial pressure in adult vertebrates including: teleosts, amphibians, reptiles, birds, and mammals. Few studies however have quantified the maturation of this critical regulatory mechanism. Those species that have been investigated during ontogeny, embryonic chickens and American alligators, display a hypertensive bradycardia which differs from that reported in adults. Our goal was to identify how ANG II function changes in a non-mammalian species, the American alligator (*Alligator mississippiensis*).
We hypothesized that juveniles would exhibit a cardiovascular response that typifies the adult ANG II response. The cardiovascular response to Ang II and the contribution of the cholinergic and adrenergic receptors to that response were investigated in embryonic and juvenile American alligators.
Ang II delivery produced a transient hypertension in both embryos and juvenile alligators. The response to Ang II appears to increase in sensitivity from embryonic to post-hatching life indicating a change in the intracellular signalling or receptor peptide affinity. However, embryos and juveniles differed in the heart rate response, as hypothesized. Embryos displayed a pronounced bradycardia, whereas juveniles responded with a tachycardia. The source of the embryonic bradycardia was attributed to secondary cholinergic stimulation. While in the juveniles the tachycardia could be attributed to β-adrenergic receptor stimulation.
Collectively, the Ang II response appears to involve a shift in the contribution of cholinergic and adrenergic receptor stimulation between embryonic and post-hatching life.

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Poster session – Friday 5th July 2013

A11.58

Effects of synthetic glucocorticoid exposure during early development in zebrafish, *Danio rerio*

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The effects of synthetic glucocorticoids on the aquatic environment have largely been overlooked, yet it is well documented that endogenous glucocorticoids play key roles during development. Prednisolone, a synthetic glucocorticoid, is a widely prescribed pharmaceutical, frequently detected within the aquatic environment. However, few studies have assessed the potential toxicity of prednisolone to early life stages of fishes.

Synthetic glucocorticoids are potent glucocorticoid receptor agonists which may allow them to mimic endogenous glucocorticoids, and specifically target physiological processes associated with these hormones during early development. This study investigated the toxicity of prednisolone during embryogenesis in zebrafish (*Danio rerio*) using morphological and physiological endpoints at environmentally relevant concentrations.

Embryos were exposed to 0.1, 1 or 10 µg/l of prednisolone immediately following fertilization for 96 hours. Length (mm), yolk sac area (mm^2) and oxygen consumption (µmol/h) were measured throughout embryogenesis (at 24, 48, 72 hpf). Background adaptation, i.e. the change in the amount of pigment dispersed within the skin in response to changing light conditions, was also recorded at 96 hpf to assess the impact of prednisolone upon the development of the eye.

No effect of prednisolone on length or yolk sac area was found. However, oxygen consumption was significantly affected. The implications of these physiological changes and the effects of synthetic glucocorticoids on response to light will be discussed.

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Poster session – Friday 5th July 2013

A11.59

Adenosinergic regulation of the cardiovascular system in the red-eared slider (*Trachemys scripta*)

William Joyce (Aarhus University, Denmark), Hans Gesser (Aarhus University, Denmark) and Tobias Wang (Aarhus University, Denmark)

Adenosine is released by cells when oxygen supply does not meet demand, and serves as an important 'retaliatory metabolite' to dilate hypoxic vascular beds and thereby restore oxygen delivery. The role of adenosinergic signalling in reptiles, however, is not well understood. Adenosine is known to protect the brain in anoxic turtles, but does not appear important for the regulation of the cardiovascular system during long-term anoxia. Indeed, early studies suggested turtles lack cardiac purine receptors, despite them being present in fishes, amphibians and mammals.

In turtles, apnoea and the coinciding cardiac shunt patterns result in temporary physiological hypoxia that may stimulate adenosine release. In six anaesthetized turtles (pentobarbital) we measured a significant 35% decrease in heart rate upon intra-arterial adenosine administration (2.5 µmol/kg). Pulmonary flow (Q_{pul}) fell significantly accompanied by a rise in systemic flow (Q_{sys}), which lead to a 40% decrease in Q_{pul}/Q_{sys}. These changes are consistent with the bradycardia and right-to-left shunt that prevails during apnoea.

In a separate *in vitro* study, we also show that adenosine elicits a dose-dependent negative inotropic effect on isolated turtle cardiac strips, further refuting earlier claims of an absence of cardiac purinoceptors in turtles. In synthesis, our results suggest adenosine can exert significant cardiovascular effects in turtles, and may contribute to regulating shunt patterns during hypoxia.

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Poster session – Friday 5th July 2013

A11.60

Autonomic control of heart rate during embryonic development of the green iguana lizard (*Iguana iguana*)

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The progressive establishment of autonomic control of the cardiovascular system during embryonic development is poorly understood in reptiles. We have studied the responses of the heart in embryos of the green iguana to injections of agonists and antagonists of cholinergic and adrenergic control and to hypoxia.

Drugs were administered by topic application onto the hearts of early embryos and by injection into the amniotic fluid of later embryos. Acetylcholine slowed or stopped the heart and this effect was antagonised by application or injection of atropine, indicating that muscarinic cholinoceptors are present on the heart of early embryos. However injection of atropine alone was without effect until immediately before hatching, when a 15% inhibitory tonus was established. This increased towards 25% in adult iguanas. Although epinephrine was without effect, injection of propranolol slowed the heart throughout development, indicating the presence of adrenergic receptors on the embryonic heart, possibly stimulated by high levels of circulating catecholamines. The calculated excitatory tonus varied between 40 and 70% until immediately before hatching when it fell to 25–30%; a level that was retained in the normoxic, inactive adult.

Hypoxia (5% oxygen) caused a bradycardia in early embryos that was unaffected by injection of atropine indicating that it was a direct effect upon the heart. In later embryos hypoxia caused an initial tachycardia that was unaffected by injection of atropine, while injection of propranolol reduced heart rate and uncovered an hypoxic bradycardia. Hypercapnia (5% CO_2) was without effect on heart rate in late stage embryos.

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Poster session – Friday 5th July 2013

A11.61

Effects of anoxia and reoxygenation on turtle (*Trachemys scripta scripta*), rainbow trout (*Oncorhynchus mykiss*), and rat cardiomyocyte contractility

Mark A Scott (University of Oslo, Norway), Holly A Shiels (University of Manchester, United Kingdom), Goran E Nilsson (University of Oslo, Norway) and Gina LJ Galli (University of Manchester, United Kingdom)

The purpose of this study was to investigate the intrinsic tolerance of freshwater turtle (*Trachemys scripta scripta*) cardiomyocytes to oxygen deprivation and subsequent reoxygenation. Since contractility in response to anoxia and reoxygenation has never been measured in turtle myocytes, or any other anoxia-tolerant animal, a comparative approach was adopted and contractility in the anoxia-intolerant rat and rainbow trout (*Oncorhynchus mykiss*) was also assessed.

Isolated cardiomyocytes were exposed to sudden anoxia for 20 minutes, followed by 20 minutes of reoxygenation. During anoxia the cells from all three species showed shortened speeds of contraction and relaxation and an overall reduced strength of contraction in comparison to normoxia. However, both the magnitude and the duration of the changes seen were comparatively minor in the anoxia-tolerant turtle. Moreover, turtle cardiomyocytes were able to return to normoxic performance within minutes of reoxygenation, whereas those from rat and trout required several times longer to recover, or never recovered.

These findings will be combined with measurements of calcium flux to illuminate the mechanism of cardiomyocyte anoxia tolerance in turtles.

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Poster session – Friday 5th July 2013

A11.62

Effects of domestication and gender on walking energetics in leghorn chickens

Kayleigh A Rose (University of Manchester, United Kingdom) and Jonathan R Codd (University of Manchester, United Kingdom)

The selective breeding of chickens has led to pronounced differences in size and posture between breeds, and all breeds exhibit male-biased (~21%) sexual size dimorphism (SSD). Little is understood, however, on the potential physiological and morphological constraints imposed by artificial selection and gender on their terrestrial locomotion.

Leghorn chickens are selected for large (~2 kg) and bantam (~1 kg) varieties. Here, we determine the effects of size and gender on the energetics of treadmill walking in leghorn chickens, using respirometry. Anatomical and morphological data were also collected to assess body size differences between groups.

We demonstrate that large and bantam leghorns shared an identical mass-specific metabolic cost of walking, which is likely a result of the isometric scaling of anatomical components between varieties. Furthermore, there were no differences in the mass-specific cost of transport between genders, despite male-biased SSD of muscles and female-biased SSD of internal organs. Males, however, were capable of higher top speeds, which is likely a result of the larger force generating capacity of their muscles. We suggest that females employ different gait kinematics to avoid the extra cost of supporting more weight with less muscle.

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Poster session – Friday 5th July 2013

A11.63

The effect of acute temperature change on the expression of behaviour in individual European minnows (*Phoxinus phoxinus*)

Dana A Weldon (University of Glasgow, United Kingdom) and Shaun Killen (University of Glasgow, United Kingdom)

Temperature is known to have an effect on the behaviour and physiology of ectothermic species, most previous studies examining the effects of temperature have compared individuals acclimated to one temperature. However, ectotherms are often exposed to acute temperature fluctuations as they move through their environment. Little is known about individual variability, behavioural responses to acute temperature changes, and how thermal sensitivity may relate to intrinsic personality traits.

We examined these issues using common minnows (*Phoxinus phoxinus*; n=36), to determine if acute temperature changes between 7, 10, and 13°C would have an effect on swimming speed (activity) and wall following behaviour (boldness). Minnows were exposed separately to three temperatures and their spontaneous behaviour was recorded for 10 minutes in an open field test.

There was a significant increase in swimming speed as temperature increased, but individual swim speed between temperatures were not correlated. Further, there was no relationship between the magnitude of the change in swimming activity and a separately assessed boldness score, suggesting that individual sensitivity to temperature change is not strongly related to intrinsic boldness. Wall following did not show a significant change between temperatures, but the degree of wall following was repeatable among individuals when at 7°C and 13°C. Individual sensitivity to temperature change as assessed by wall following was also highly variable.

Overall, these results suggest that personality traits such as boldness do not have a major influence on thermal sensitivity in this species. It is possible that thermal sensitivity may vary in relation to other intrinsic traits.

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Poster session – Friday 5th July 2013

A11.64

Is a peaceful fish a sign of improved animal welfare or the adverse effects of an opioid drug?

Albin Gräns (University of Gothenburg, Sweden), Erik Sandblom (University of Gothenburg, Sweden), Anders Kiessling (Swedish University of Agricultural Sciences, Sweden) and Michael Axelsson (University of Gothenburg, Sweden)

The use of fish models in biomedical research increases. Since behavioural and physiological consequences of surgical procedures may affect experimental results, these effects should be defined and, if possible, ameliorated. Therefore, the use of analgesia or 'painkillers' should be considered after invasive procedures also in fish, but presently, there is little information on the effects of analgesics on fish. This study assessed the effect of an opioid drug, buprenorphine (0.05 mg/kg), on resting heart and ventilation rates during 7 days of postsurgical recovery in rainbow trout (*Oncorhynchus mykiss*) at 10°C. Both variables were recorded non-invasively by measuring the bioelectric potentials generated by the fish in the water. Buprenorphine significantly decreased both heart rate (562 beats min^{-1}) and ventilation rates (4±1 breaths min^{-1}) and the effects were most pronounced at 4–5 days after anaesthesia, surgical procedures and administration of the drug. In fact resting heart and ventilation rates in fish given buprenorphine were to our knowledge the lowest values ever reported for rainbow trout (21.9–2.6 beat min^{-1} and 40.0±1.7 breaths min^{-1}). Somewhat surprisingly the same effects of buprenorphine were seen also in the two control groups which had not been surgically wounded. These results indicate that the reductions in heart and ventilation rates are not caused by an analgesic effect of the drug, but may instead be a sedative effect which one of many known adverse effects of opioid drugs. Thus, before using buprenorphine for fish models in future biomedical research, these potential adverse effects need to be further characterized.

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Poster session – Friday 5th July 2013

A11.65

Kidney compensates ion losses in copper-exposed exercised shubunkin (*Carassius auratus auratus*)

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Copper toxicity causes gills ionoregulatory and respiratory dysfunction in freshwater fish. However, not much is known about the effect on kidney. This research was conducted to investigate sublethal Cu effects on the ionoregulation in both gill and kidney of shubunkin, *Carassius auratus auratus.* Fish were not fed and exposed to 0 µmol/L (N=16, control), 0.34 µmol/L (N=16) and 0.84 µmol/L (N=16) of copper ($CuSO_4$ $5H_2O$) for 24 or 168 hours. An additional fed control group (N=8) was sampled to reveal the effect of food deprivation alone. All fish were exercised to exhaustion prior to sampling. Food deprivation alone had no effect on ionoregulation; and swimming performance (measured as critical swimming speed) was not affected by either fasting or Cu exposure. Low plasma osmolality level was noticed in fish exposed to the low Cu level for 168 hours. Cu induced a dose dependent Na^+ loss in all Cu exposed groups. Both gill Na^+/K^+ ATPase and H^+ ATPase (ATPase) activities were largely undisturbed. Except when fish were exposed to the high Cu level for 168 hours, where these ATPase activities were down-regulated and remarkable Na^+ lost. Interestingly, both kidney ATPase activities were up-regulated when challenged with Cu. These ATPase activities were increased more prominent in fish exposed to the high Cu level. At both Cu levels, kidney ATPase activities were up-regulated likely act as a compensatory strategy to enhance Na^+ reabsorption. However, this up-regulation was not sufficient to restore Na^+ to control levels in the highest exposure group.

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Poster session – Friday 5th July 2013

A11.66

Maintained mRNA and protein expression of H_2S-producing enzymes in anoxia-tolerant vertebrates during anoxia and reoxygenation

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The gasotransmitter hydrogen sulphide (H_2S) protects against ischemia/reperfusion injury in cardiac and cerebral tissue of anoxia-intolerant mammalian species by promoting ROS scavenging, antioxidant formation and anti-apoptotic responses. For anoxia-tolerant vertebrates, previous study has suggested a role of H_2S in mediating the cardiovascular responses to anoxia. To further examine the potential role of H_2S in facilitating anoxia-survival of anoxia-tolerant vertebrates, we measured the transcript and protein abundance of the three primary H_2S-synthesizing enzymes (CBS, CSE and 3MST) in the heart and brain of normoxic, anoxic and reoxygenated freshwater turtles (*Trachemys scripta*) and crucian carp (*Carassius carassius*). We hypothesized that if H_2S was critical for anoxia survival, expression of the enzymes would remain unchanged or be up-regulated with anoxia and/or reoxygenation. Indeed, for both species, transcript and protein expression of the enzymes were largely maintained with prolonged anoxia exposure (24 hours at 21°C and 14 days at 5°C, turtle; 5 days at 10°C, crucian carp). Only a minor decrease of CBS mRNA was observed in the crucian carp brain with anoxia, which subsequently returned to the control normoxic level upon reoxygenation. With reoxygenation, 3MST was increased significantly at the protein level in the turtle heart at 5°C and at the mRNA level in the crucian carp brain. The maintenance of mRNA and protein expression of the H_2S-producing enzymes with anoxia exposure, as well as the up-regulation of 3MST with reoxygenation, suggest that H_2S may play an important role in mediating anoxic survival of the two champions of vertebrate anoxia survival.

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Poster session – Friday 5th July 2013

A11.67

Metabolic cold adaptation in *Drosophila*

Branwen Messamah (Aarhus University, Denmark), Hans Malte (Aarhus University, Denmark), Volker Loeschcke (Aarhus University, Denmark) and Johannes Overgaard (Aarhus University, Denmark)

Metabolic cold adaptation (MCA) is the phenomenon whereby cold climate species display an elevated metabolic rate (MR) relative to their warm climate sister species at the same trial temperature. Essentially, MCA predicts the conservation of the rate of temperature-dependent biological processes (overall estimated from MR) in cold adapted species facing lower environmental temperatures. This controversial theory has received mixed support in the literature, but appears to be present as a general trend among insect species where it has been detected across a wide range of orders. The present study aims to examine MCA within a narrower phylogenetic context, using over 50 species from the Drosophilid family. These species contain representatives from a wide range of latitudes and environmental conditions spanning temperate and tropical habitats. All species are reared under identical conditions and MR is subsequently measured at 10 and 20°C; using intermittent closed-system respirometry. This system allows several independent measurements of oxygen consumption rate and activity at each trial temperature, providing reliable measurements of resting MR. Preliminary data suggest no correlation between mass-specific MR and latitude or annual mean temperature. This lack of correlation is present regardless of sex and trial temperature, and remains present with or without controlling for the effects of size on the MR.

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Poster session – Friday 5th July 2013

A11.68

Oxidative stress as biomarker of ketamine-induced toxicity during early zebrafish development

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Increasing concern has been raised since the occurrence of pharmaceutical active compounds in the aquatic environment was reported. These have been recognized as emergent contaminants. Ketamine is one of such compounds that has been detected in wastewater and surface waters. We have previously reported ketamine toxic effects in zebrafish during early development. The present work intends to use oxidative stress parameters as biomarkers of ketamine-induced zebrafish embryo toxicity.
Zebrafish (*Danio rerio*) embryos, 2–3 hpf-hours post fertilization, were exposed during 20 minutes to 0.0, 0.2, 0.4 or 0.8 mg/L concentrations of ketamin. Four exposure replicates were done (125 embryos/group). By 8 hpf, oxidative stress markers, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), lipid peroxidation (TBARS) and protein damage (carbonyl protein content), were evaluated using conventional methods. A one-way ANOVA followed by Tukey's test (p=0.05) was performed. The results showed that 20 minutes of ketamine exposure decreased, not significantly, SOD and GPx activities. CAT activity showed a tendency to increase in a concentration-dependent manner. Despite a tendency to increase, no statistical differences were observed in the content of TBARS neither on the protein damage.
Our results show that ketamine may interfere with antioxidant responses in zebrafish and that oxidative stress parameters might be useful biomarkers of ketamine-induced toxicity during early zebrafish development.
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Poster session – Friday 5th July 2013

A11.69

Many kinds of cold: Evaluation of different measures of insect cold resistance

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The ability to tolerate low temperature is one of the most important factors determining the distribution of ectothermic species. According to the commonly used nomenclature, Drosophilids belong to freeze avoiding species, but in reality most Drosophilids die or become injured at temperatures considerably above that causing freezing. Over the years several studies have developed different methodologies to characterize species and population specific 'chill tolerance' but few have thoroughly investigated how these measures relate to each other and how well they describe differences in distribution patterns. In the present study we categorize 12 species with markedly different distribution patterns ranging from the tropics to a cold temperate climate. Species are currently being characterized using five of the most common measures of cold resistance: chill coma temperature (CT_{min}), the temperature at which the insect loses neuromuscular function and stops responding to stimuli; chill coma recovery time, the time before the insect regains neuromuscular control, after subjection to cold; LT_{50} (time), the time it takes to reach 50% mortality under exposure to a constant low temperature; LT_{50} (temp), the temperature at which 50% mortality is reached after cold exposure for a set time period; and supercooling point, the average temperature of spontaneous freezing in the insect subjected to a gradual cooling at a rate of 1°C min^{-1}. The findings are discussed in relation to the underlying physiological mechanisms as well as in relation to their validity to describe important ecological adaptations.

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Poster session – Friday 5th July 2013

A11.70

Characterization of the salt secreting organ of the stripped marine catfish *Plotosus lineatus*

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The Plotosidae catfishes are one of the few catfish families with marine, brackish water and freshwater members. They are also unique amongst the teleosts in possessing a specialized salt secreting organ, the dendritic organ (DO). In contrast, salt secretion in all other marine teleosts is typically preformed by gill ionocytes. The evolution of this specialized ionoregulatory organ likely permitted the successful habitation of marine environments by Plotosidae catfishes.

This study was directed at the molecular characterization of the DO ion transport mechanisms in *Plotosus lineatus*, the stripped marine catfish, and contrasted the expression pattern of this salt secreting organ with the other main teleost ion transporting organ, the gill.

Na^+/K^+-ATPase (NKA) activity was 4.5x higher in DO in contrast to gill which correlated with immunoblot (IB) data for NKA α subunit and immunohistochemical localization of NKA-immunoreactive (NKA-IR) cells (ionocytes). In the gill there were few NKA-IR cells present whereas the parenchymal cells of the DO showed strong expression. The secretory $Na^+:K^+:2Cl^-$ cotransporter (NKCC1) expression in gill was not detectable in contrast to DO, which displayed high levels (IB and IHC). Cystic fibrosis transmembrane conductance regulator (CFTR) was detected in DO but not gill by IB, however, it was not possible to confirm these results by IHC.

Taken together, these results indicate that there is a clear shift of NaCl secretion mechanisms from the gills to the DO of *P. lineatus* firmly establishing this organ as key for hypo-osmoregulation.

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Poster session – Friday 5th July 2013

A11.71

A tribute to Jeff Graham: A giant of research on comparative respiratory physiology and transitional species

Martin Tresguerres (Scripps Institution of Oceanography, UCSD, United States), Jeffrey B Graham (Scripps Institution of Oceanography, UCSD, United States), Corey J Jew (Scripps Institution of Oceanography, UCSD, United States), Nicholas C Wegner (Southwest Fisheries Science Center, NOAA Fisheries, United States), Troy Baird (University of Central Oklahoma, United States) and Kathryn Dickson (California State University Fullerton, United States)

From Jeff Graham: "The essence my career has been a focus on comparative respiratory biology and its related variables such as metabolism and body temperature. Added to this is an emphasis on species or groups characterized as being 'transitional' between the normal life history and physiology of the group at large. Examples include the sea snake *Pelamis*, which has 'returned to the sea' and, unlike the majority of sea snake species never leaves the water. Another is the tunas, which are considered to be 'super fish' because of their many adaptations for elevated aerobic capacity and their extensive migrations that distinguish them from most other epipelagic fishes. Finally there are the air-breathing fishes, a diverse assemblage of species occurring among 50 families of osteichthyans." From TB, CJ, NW, KD and MT: "In addition to his outstanding scientific contributions, Jeff was a wonderful mentor, colleague, and friend, and he unveiled the satisfaction and fun of studying the natural world. He was never short on sharing wisdom, humour, and inspiration, and was a strong influence on those around him. Jeff mentored a large number of students and postdocs, many of which have become prominent scientists. Furthermore, he encouraged his students to interact across generations, and our current connections are a testament to that. Jeff's mentoring activities are certainly an important component in his even larger legacy."

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Poster session – Friday 5th July 2013

A11.72

Structure and Na^+, K^+-ATPase immunolocalization in the midgut and hindgut of the shrimp *Litopenaeus vannamei*

Saber Khodabandeh (Tarbiat Modares University, Iran), Mahyar Zare (Khazar University, Iran), Saeid Voshtani (Khazar University, Iran) and Ferial Monsef (Tarbiat Modares University, Iran)

Although previous reports indicate that the cells of various portions of the crustacean gut are typical of salt-transporting absorption or secretory epithelia, there is not more information about ion regulatory function for the shrimp gut by Na^+/K^+-ATPase, a key enzyme which uses ATP as source of energy. Gut structure and Na^+/K^+-ATPase immunocalization were studied in cultured *L. vannamei* (from saline water), through immunofluorescence light microscopy using a mouse monoclonal antibody $IgG_{\alpha 5}$.

Our results showed that, the midgut epithelium was lined by a folded and simple epithelium that possesses columnar cells. In the posterior end of midgut a dorsal opening leads into the posterior diverticulum, and the rectum lined by six pad-like rides appears.

Na^+/K^+-ATPase immunofluorescence were detected in the basal part of the cells of the midgut cells with an increasing immunostaining from anterior to the posterior parts. In the rectum ride cells, this immunofluorescence observed in the all cells and particularly in the basolateral parts of lumen side epithelial cells. The presence of Na^+/K^+ATPase with a good intensity in the midgut and hindgut of the shrimp *L. vannamei* could support the idea that beside its implication in nutrient absorption, their cells are also involved in water and ions regulation.

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Poster session – Friday 5th July 2013

A11.73

Venom ducts structure and NKCC localization in *Conus textile* from the Persian Gulf

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Predatory marine snails of the genus *Conus* use their special venom for different purpose like feeding, defence and competition. This venom produces by venom apparatus that have three main parts; venom bulb, proximal duct and distal duct. Venom ducts structure and Na^+-K^+-$2Cl_2^-$ co-transporter immunolocalization were studied in *Conus textile* through immunofluorescence light microscopy using a mouse monoclonal antibody T_4.

Light microscopy reveals three distinct zones in venom duct: an external layer is built up of two layers: an external layer of longitudinal muscular fibres and connective tissue, and an internal layer of connective tissue and circular muscular fibres. The internal epithelial layer showed very elongated columnar cells with basal nuclei. The apical parts of cells were filled with several granules, and more intense of these granules were observable into the duct lumen. The columnar cells basal part showed a good immunofluorescence of NKCC and this fluorescence also observed in the membrane of granules. Venom bulb showed two rows of the circular and contractions muscles, with a narrow lumen. Their lumen epithelial is composed of the cuboidal to plat cells. No immunofluorescence of NKCC was observed in the venom bulb cells. Small granules with weak immunofluorescence membrane are also seen into the lumen.

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Poster session – Friday 5th July 2013

A11.74

Aquaporin localization and expression in the kidney of Atlantic salmon during smoltification and seawater acclimation

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In addition to the gills and intestine, the kidney is a primary osmoregulatory organ of the anadromous Atlantic salmon. In FW, it produces large amounts of dilute urine and conserves important salts and nutrients by reabsorption in the proximal and distal segments of the nephron. In SW, urine production is highly reduced and the role of the kidney is to conserve water and to secrete excess divalent ions.
As established in mammals, water transport across the renal epithelia may be mediated by aquaporins (Aqps), and we hypothesized that renal Aqp expression patterns change during smoltification and SW acclimation in order to accommodate the major functional reorganization of the kidney. Immunohistochemistry showed apical and basolateral localization of Aqp1aa, sub-apical localization of Aqp1ab and basolateral localization of Aqp8b in specific tubule segments, confirming a route for transcellular water transport in both FW and SW nephrons. We analysed mRNA expression levels of multiple Aqp paralogues during smoltification and found decreasing trends in transcript abundance of aqp1aa, aqp1ab and aqp3a1 around the peak of smoltification in FW.
In response to SW transfer, aqp3a1 transcript abundance significantly increased in both parr and smolts. In contrast, transcript levels of aqp1aa and aqp1ab decreased following transfer to either SW or FW suggesting a general effect of transfer stress.
These results indicate that aquaporins are important in regulating kidney function during SW acclimation and smoltification, which may be important for maintaining overall hydromineral balance.

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Poster session – Friday 5th July 2013

A11.75

Swimming against the odds: The unusual swimming kinematics of the ocean sunfish

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Fishes show a wide diversity of swimming modes. In addition to being the world's largest teleost fish, the ocean sunfish (*Mola mola*) possesses an unusual swimming style based on beating their greatly enlarged dorsal and anal fins. The unusual body shape of these fish also includes a modified caudal fin that acts as a rudder to control direction while swimming. Here we describe the relationship between fin-beat frequency and swim speed in ocean sunfish.
Two individuals were filmed in three-dimensions during routine swimming in a captive pelagic tank (33 x 22 x 8 m) using synchronised stereo cameras. In addition to using synchronous beats of the dorsal and anal fins to propel the fish forward, individuals also use these fins to rotate sideways, and to assist the caudal fin in controlling directionality. Ocean sunfish use powerful beats of their rudimentary tail to generate forward thrust when swimming at very fast speeds during feeding, demonstrating an additional function of the caudal fin beyond use as a rudder.
Together these results suggest that ocean sunfish are active swimmers capable of high swimming speeds in the pelagic environment.

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Poster session – Friday 5th July 2013

A11.76

Biochemical changes during brooding and pelagic development in larvae of European flat oyster *Ostrea edulis*

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European flat oysters *Ostrea edulis* are larviparous, and brood their larvae for a period before they are released. Thus, both the biochemical reserves of the egg and larval diet are known to play an important part in larval nutrition and survival. This study aimed to investigate the contribution of maternal diet, diet inside the brood chamber and larval diet post-release, on the biochemical composition of eggs and pre- and post-release larvae, as well as larval growth rate and survival to metamorphic competence. Groups of broodstock were held for 1 month to 1 year on a diet of cultured microalgae (*Isochrysis aff. galbana* clone T-iso; ISO) and *Chaetoceros gracilis*, and compared to broodstock brought in directly from the wild during the reproductive season. Samples of eggs, brooded larvae, and naturally-released veliger larvae were collected, measured and processed for proximate composition (total lipid, total protein, total carbohydrate, organic and inorganic content), and fatty acid composition.
In addition, larvae from one broodstock group were given three different microalgal diets composed of 100% ISO, 70% ISO + 30% *Chaetoceros calcitrans* (*CC*), or 50% ISO + 30% *CC* + 20% *Tetraselmis chuii* all at the same ration (75,000 cells mL^{-1} equivalent dry weight of ISO). Growth and survival were measured every 2–3 days until metamorphic competence, and large differences were observed in both growth and survival between diets. The relationship between broodstock and larval diets, development stage and biochemical composition will be discussed.

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Poster session – Friday 5th July 2013

A11.77

Effect of sodium nitrite intoxication on the cerebellar morphology in mature and aged rats

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Sodium nitrite ($NaNO_2$) has numerous applications in food and chemical industry, medicine, etc. However, the overdose of $NaNO_2$ may cause inflammation and hypoxia in the CNS, especially risky for the aging brain. Cerebellar morphological changes following $NaNO_2$ intoxication has not been studied in details although there are data showing a high vulnerability of Purkinje cells to hypoxic insult. The aim of the present study is to examine the effect of $NaNO_2$ intoxication on the cerebellar morphology in mature and aged rats. Four and 12 month-old male Wistar rats were injected intraperitoneally with 50 mg/kg bw $NaNO_2$ and sacrificed at different time intervals (1h, 5h, 24h, and days 2, 10, 20) following the administration. Cerebellum was studied histologically using silver-copper impregnation for neurodegeneration. Histopathological studies of both mature and aged rats demonstrated widening of the perineuronal spaces, vacuolization and a loose granular layer. Enhanced microvascularization and hyperaemia were also observed. These changes were most prominent in the aged rats. They also demonstrated highly impregnated impaired neuronal processes and an unusual dense and tangled appearance of the basket cell axonal plexuses surrounding the Purkinje cell soma. Damaged neuronal processes and single vacuoles were visible in the control aged brain as well. However, they were much less pronounced than in the treated rats. Our findings demonstrate that the cerebellum of aged rats is more susceptible to the effect of $NaNO_2$ as compared to mature rats. Thus, $NaNO_2$ intoxication accelerates the manifestation of the neurodegenerative changes concomitant with the normal brain aging.

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Poster session – Friday 5th July 2013

A11.78

Regulating cell proliferation in the developing zebrafish: The role of the CCAAT/enhancer-binding proteins

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Many genes that regulate cell fate processes also play critical roles during vertebrate embryonic development. The CCAAT/enhancer-binding protein delta (C/EBPδ) is a highly conserved transcription factor capable of regulating numerous cell fate processes, such as cell growth, differentiation, proliferation and apoptosis. C/EBPδ is inducible during cellular stress responses, including inflammation and responses to growth factor deprivation or thermal stress. C/EBPδ is stress-inducible in a diversity of fishes, including the zebrafish *Danio rerio*; however, little is known about its role in fish development. To elucidate a developmental role for C/EBPδ, we generated transgenic C/EBPδ-overexpressing zebrafish (C/EBPδ++). We found that overexpression of C/EBPδ leads to severe developmental defects, including reduced body length, oedema, liver malformation and retinal abnormalities. The proportion of individuals that display developmental abnormalities is significantly greater in C/EBPδ++ embryos compared to control embryos. In addition, C/EBPδ overexpression significantly reduces survival of larvae over time. TUNEL analysis suggests C/EBPδ++ embryos exhibit an increase in apoptotic cell death compared to control embryos. These data suggest a critical role for C/EBPδ in numerous developmental processes, including promoting programmed cell death during development. Mutations in C/EBPδ have been implicated in the progression of human tumours, including those of myeloid, hepatocellular and breast cancers. Therefore, our C/EBPδ++ zebrafish will serve as a valuable model for examining the function of this gene during development, as a part of the cellular response to stress and in pathological states such as tumour progression. On-going studies are aimed at the alpha and beta isoforms of this gene family.

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Poster session – Friday 5th July 2013

A11.79

Survival pattern of bank voles selected for high aerobic capacity: Testing the 'rate of living' theory

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Ageing is an issue of interest in evolutionary biology, ecology, physiology, and medicine. According to the 'rate of living theory', animals with high metabolic rate have increased ageing and mortality rates. Our artificial selection experiment on bank voles (*Myodes*=*Clethrionomys glareolus*), based on four lines selected for high swim-induced aerobic metabolism (A) and four unselected-control lines (C), provides a suitable model to test the hypothesis. Voles from the selected lines achieved about 50% higher rate of the maximum swim-induced oxygen consumption, and had also significantly increased basal metabolic rate and food consumption. In generation 13, we monitored survival in 2,115 young animals (A: 1112, C: 1003) from 17th to 75th day of life (from weaning to sexual maturity) and in 167 adults (A: 82, C: 85) from 75th day to advanced senility (534 days). Statistical analyses were based on proportion of survived animals in each of the eight lines. The survival did not differ significantly between lines either in young animals (mean±SD; A: 0.959±0.03, C: 0.978±0.01; Mann-Whitney U=2.000, p=0.083) or in adults (A: 0.776±0.14, C: 0.762±0.14; Mann-Whitney U=8.000, p=1.000). Thus, contrary to the expectation of 'the rate of living theory', selection for high aerobic metabolic rate did not influence age-related survival. In the next step of the project we ask how the selection affected age-related changes in physiological performance (forced-exercise aerobic metabolism, running speed) and underlining biochemical traits (mitochondria quantity, oxidative damage, and antioxidative enzymes).

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Poster session – Friday 5th July 2013

A11.80

Contribution of engrailed-expressing Johnston's organ neurons to the auditory response of *Drosophila melanogaster*

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Engrailed is an important neuronal transcription factor in all animals, and has an additional well-known role in segment formation in insects. However, its role in neuronal determination is less well understood. Recently it was shown that a subset of Johnston's Organ neurons (JONs) in *Drosophila* express engrailed through to adulthood. Engrailed-expressing JONs were localized using *en-GAL4* to drive *UAS-CD8GFP*, and also using antibodies against the engrailed protein and its paralogue, invected. Only two engrailed and invected expressing JONs are located in the medial part of the pedicel – the majority are situated in the posterior. If the current model of JO function is correct, alternating activation of medial and posterior groups of JONs results in the typical antennal nerve response that has peaks at twice the sound frequency. In this case, silencing of En-expressing JONs in the posterior group should result in the specific reduction of only one set of peaks. We recorded sound-evoked potentials (SEPs) from the antennal nerve and silenced En-expressing neurons using several methods, including expression of an inward rectifying potassium channel, Kir, and by expression of the modified influenza toxin, M2(H37A). We found that, contrary to the established model, En-JON silencing affects both sets of peaks to an equal extent, suggesting that all JONs respond to backwards and forwards antennal movements. Comparison with other subsets of JONs labelled with other *GAL4* lines confirms this finding. A revision of the anatomy of the pedicel–funicular joint suggests how this could be possible.

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Poster session – Friday 5th July 2013

A11.81

Retinoic acid signalling system in developing and adult neurons

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Retinoic acid (RA), an important signalling molecule and a member of the retinoid family, is a potent transcriptional activator that plays roles in the regulation of neuronal differentiation and in retinal patterning during development, and which may be involved in neuronal plasticity and regeneration in the adult brain. RA is synthesized by the enzyme retinaldehyde dehydrogenase (RALDH), the cellular retinoic acid binding proteins (CRABP-I and CRABP-II) regulate its availability, and its various effects are mediated via the retinoic acid receptors (RARs). The purpose of this study is to study the distribution of the different components and understand RA's effects on the process of axonal regeneration and neurite outgrowth in developing and adult neurons. RALDH, RARs, and CRABPs are present in neurons of the normal adult visual system and their protein levels are significantly increased at the time when regeneration is occurring after optic nerve transection. Brain derived neurotrophic factor treatment up-regulates RARs levels in regenerating retinal ganglion cells (RGCs). RA actions on neurite extension are also studied in different populations of neurons during development and in vitro with specific agonists and antagonists of RA signalling. Our results are consistent with the idea that adult neurons may reuse developmental signalling mechanisms in response to axonal injury.

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Poster session – Friday 5th July 2013

A11.82

The effect of *Artemisia aucheri* flowering taps allergenicity on Guinea pigs

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The term allergy, is the series of events which occurs when an antigen, which is not harmful in itself, causes an immune response, leading to disorder and disease. Therefore, the aim of this study was to evaluate of the allergenicity effect of *Artemisia aucheri* extract. In this research, flowers of *A. aucheri* were collected from Isfahan around, Iran in September 2012. Flowers were extracted using phosphate-buffered saline, pH 7.4 and 80% ethanol. Three concentration of extracts (5%, 10%, and 15%) were prepared. The experiments were intraperitoneally injection of the extracts three times, once every 10 days. The flowering taps allergenicity were detected using subcutaneous, skin scratch, serological and clinical tests. Tests were done on Hartley male Guinea pigs. In treated and control groups the appearance of wheal, their diameter, serum IgE, eosinophilia, neutrophilia were compared. Means of triplicate measurements and standard errors were determined for each sample. Dates were analysed using ANOVA test in the $p<0.05$.
During the skin prick test, the maximum allergenic sensitivity was observed for 15% ethanolic extracts, with an average wheal diameter of about 3 cm. The numbers of eosinophils and neutrophils were increased in the treated animals with extracts comparing control groups. The serum of treated animals with phosphate-buffered saline and 80% ethanol extracts contained more IgE than serum of control animals. Our results confirm that *A. aucheri* is another allergenic plant. The confirmation of these aspects would facilitate the preparation of an effective extract, improving the diagnosis of the allergy to the *A. aucheri*.

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Poster session – Friday 5th July 2013

A11.83

Cytotoxic effects of n-butanolic fraction of *Achillea wilhelmsii* C. Koch flowers on the HT29 cell line

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Natural products, especially plants have been used for the treatment of various diseases such as cancer for thousand years. The *Achillea wilhelmsii* C. Koch belongs to the family Compositeae and is widely found in different parts of Iran.
In this project, *A. wilhelmsi*i have been collected from around Sharekord and extracted by fraction n-butanol and were assayed on HT29 cell line (colon cancer). HT29 cell line was purchased from the National Cell Bank of Iran, Pasture Institute of Tehran. The concentrations 12.5, 25, 50, 100 µg/ml of fraction n-butanolic were prepared and measured by MTT method. The results analysed by using SPSS 18 and ANOVA one way.
The results in this study were showed that the difference of the fraction n-butanolic of *A. wilhelmsii* flowers at concentration 25, 100 µg/ml with control group were significant. Final findings of cytotoxicity experiments were showed mentioned herbal extract in inducing cytotoxicity on HT29 cell line. Thus, this study showed that saponin compounds have a cytotoxic effect on the HT29 cell line.
Keywords: *Achillea wilhelmsii*, cytotoxic, saponin, HT29.

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Poster session – Friday 5th July 2013

A11.84

Maximum cold-induced food consumption in bank voles selected for high swim-induced aerobic capacity: Implications for the evolution of endothermy

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To test a hypothesis that high capacity for energy assimilation in cold-exposed endotherms could evolve as a correlated response to selection for increased locomotor-related aerobic capacity, we studied bank voles (*Myodes*=*Clethrionomys glareolus*) from lines selected for high swim-induced aerobic metabolism (A) and unselected control lines (C). After 13 generations, voles from the four A lines had about 50% higher aerobic capacity, 12% higher basal metabolic rate, and about 7% higher routine food consumption rate than voles from the four C lines. Body mass of the animals (mean age ± SD: 114±7.6 days, n=96) maintained in room temperature (+20°C) was higher in A (LSM6SE; 24.9±0.4 g) than C lines (22.6±0.6 g, $p=0.007$). The maximum cold-induced rate of food consumption, achieved after gradual exposure to low ambient temperatures (overall 46 days from +20°C down to -16°C or to maintain positive body mass balance), was significantly higher in A than C voles (mass-independent LSM ± SE; A: 12.19±0.18 g/day, C: 11.51±0.20 g/day, $p=0.044$). The coefficient of digestibility did not differ significantly between the lines (A: 72.83±0.53%, C: 72.21±0.57%, $p=0.527$), so the difference in food consumption reflects a difference in energy budgets. Although A have higher basic costs of maintenance, during the extreme cold exposure they maintained body mass as well as C (A: -0.41±0.22 g/day, C: -0.32±0.22 g/day, $p=0.389$). The results indicate that selection for increased locomotor-related aerobic capacity could be indeed a factor behind the evolution of high capacity for energy assimilation during prolonged cold-exposure.

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Poster session – Friday 5th July 2013

A11.85

Genotoxic effects of rocket propellant's components on rodents from natural populations

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The results of field work in the fall region of burned-out rocket stages in Russia and Kazakhstan indicate the presence of a propellant's component unsymmetrical dimethylhydrazine (1,1-DMH) and its oxidation product nitrosodimethylamine (NDMA) in soil, water and plants, concentrations exceed the MAC. The results of cytogenetic studies established chromosomal instability in *Citellus pygmaeus* Pallas and *Mus musculus* L., a common rodent species, from the area's prone rocket and space activities. This analysis indicates the presence of genotoxic factors in their natural habitat. Rats treated with 1,1-DMH and its oxidation product NDMA showed genotoxic effect. Increased mutation has been found in animals when exposed to 1,1-DMH and NDMA. The picture of structural mutations the same for wild and experimental animals. The liver LHP level of *C. pygmaeus* Pallas and *M. musculus* L. were 1.7 and 1.9 times ($p<0.01$), while MDA content were 1.6 and 1.8 times ($p<0.01$), respectively higher than the animals of the control area. MDA accumulation in the liver of experimental animals occurred by reducing the activity of antioxidant enzymes, particularly catalase. It is known that the metabolism of 1,1-DMH and NDMA forms nitrogen oxides, binding to the active site of the enzyme reduces its catalytic activity. The result of the fall in catalase activity was accumulated peroxide in the liver and strengthens the process of lipid peroxidation. Therefore, the results indicate enhancement of free-radical processes, caused by toxic factors in the environment, in particular rocket propellants.

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Poster session – Friday 5th July 2013

A11.86

RAD sequencing identifies a sex-linked SNP marker in the salmon louse (*Lepeophtheirus salmonis*)

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The salmon louse (*Lepeophtheirus salmonis*) is a parasitic copepod that incurs significant costs to the Atlantic salmon (*Salmo salar*) industry. Salmon lice are gonochoristic and normally show sex ratios close to 1:1. While this observation suggests that sex determination in salmon lice is genetic, with only minor environmental influences, the mechanism of sex determination in the salmon louse is unknown. This paper describes the identification of a sex-linked Single Nucleotide Polymorphism (SNP) marker, providing the first evidence for a genetic mechanism of sex determination in salmon lice. Restriction site-associated DNA sequencing (RAD-seq) was used to isolate SNP markers in a laboratory-maintained salmon louse strain. A total of 85 million raw sequence reads produced 281,838 unique RAD markers. RAD marker Lsa101901 showed complete association with sex for all individuals analysed, being heterozygous in females and homozygous in males. Using an allele-specific PCR assay for genotyping, this SNP association pattern was further confirmed for two unrelated salmon louse strains, displaying complete association with phenotypic sex in a total of 96 genotyped individuals. The marker Lsa101901 was located in the coding region of the prohibitin-2 gene, which showed a sex-dependent differential expression, with mRNA levels determined by RT-qPCR about 1.8-fold higher in adult female than adult male salmon lice. In summary, results of this suggest that sex determination in the salmon louse is genetic and follows a female heterozygous system. Marker Lsa101901 provides a tool to determine the genetic sex of salmon lice, and could be useful in the development of control strategies.

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Poster session – Friday 5th July 2013

A11.87

The effect of *Artemisia deserti* flowering taps extract on liver in Wistar male rats

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The liver, is a necessary organ present in vertebrates and some other animals. Liver transaminases (AST/ALT) are biomarkers of liver damage. Also, HDL and LDL are from the groups of lipoproteins which allow lipids to be transported within the blood flow. Multiple plants are used for the treatment of liver diseases. The aim of this study is to study the effects of *Artemisia deserti* extract on liver. *Artemisia* is a large, diverse genus. In this study after collecting and provision plant, they were dried under shade and ground into fine powder using electric blender, then, 20 g of flower powder were extracted with 150 mL 80% ethanol by Soxhlet extraction for eight hours. The dried extracts were stored at 4°C until used. The animals were divided into three groups. Group 1 was injected with saline, group 2 and 3 were injected with extract 100 mg/kg and 200 mg/kg respectively. The animals were anaesthetized and the AST, ALT, ALP, LDL and HDL were assayed. The liver tissue was separated and pathological changes were studied. No significant changes in liver enzymes were observed in the three groups (p>0.05). But, the flower extract of *A. deserti* cause changes in the liver tissue. The results are shown that the histopathological change probably due to the existing of artemisin and thujone at extracts. It also seems liver disorders is resolved with time.
Keywords: *Artemisia deserti*, extract, liver.

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Poster session – Friday 5th July 2013

A11.88

Mixture effects of anti-sea louse agents in *Daphnia magna*

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Sea lice (Copepoda: Caligidae) are ectoparasites of marine fish causing considerable problems in salmon farming. While different alternative approaches to sea louse control are currently being investigated, control on fish farms still relies heavily on the use of chemicals. Among marine biota, crustaceans have been shown to be particularly susceptible to non-target effects of salmon delousing agents.
In order to gain basic information regarding potential mixture effects of sea louse control agents in crustaceans, the *Daphnia magna* immobilization test was used. The organophosphates malathion and azamethiphos and the pyrethroids deltamethrin and cypermethrin were tested individually and in all pair-wise combinations. The effects of each combination were established by testing a dilution series of a binary mixture of the compounds at a fixed concentration ratio corresponding to the ratio of the median effective concentrations (EC_{50}) of the two compounds. Isobologram analysis was used to compare observed data with predicted effects based on the model of concentration additivity.
No clear relationship was found between the class affiliation of the chemicals in the mixture and the outcome of the analysis. In most binary mixtures, toxic effects of the two constituents were additive. In contrast, combination effects of deltamethrin and malathion were less than additive, while those of deltamethrin and cypermethrin were more than additive.
The results demonstrate that anti-sea louse drugs can show significant combination effects in crustaceans, and suggest that estimates of effect levels based on the model of concentration additivity may be protective in most cases.

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Poster session – Friday 5th July 2013

A11.89

Is oxygenation rate of human erythrocytes limited by membrane resistance?

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The rate of oxygenation of human erythrocytes after a step increase in the external oxygen partial pressure has been measured a number of times, primarily by stopped flow and continuous flow techniques. Early attempts of modelling the process seemed to leave little or no room for the membrane as a resistance to the oxygen transfer. Instead, the resistance was ascribed to reaction and diffusion inside the cell. However, the thin layer technique, when equilibrating a layer of whole blood, only one cell thick, has shown much faster equilibration times of approximately 10 ms. This is faster than previous model prediction on a naked layer of haemoglobin and indicates that these models are inadequate.
In this study I model the uptake of oxygen in a unicellular layer, as well as in the single erythrocyte, using a three-dimensional model that takes into account simultaneous reaction and diffusion of O_2 and haemoglobin inside the cell. It is shown that the naked single cell will equilibrate in less than 5 ms if there infinite reaction velocity and in 6 ms when a realistic, finite reaction rate is used. The addition of water between the cells brings the theoretical equilibration time closer to the measured and thus reduces the room for a membrane resistance in the transport pathway. By the use of a geometrically simpler model, it is estimated that, if the membrane itself is impermeable, it will require more than 106 aquaporin sized channels to account for the low membrane resistance.

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Poster session – Friday 5th July 2013

A11.90

Study of the allergenicity effect of *Artemisia deserti* flowering taps extract on Guinea pigs

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Allergies, known as oversensitive reactions, occur when the immune system over reacts to material. The most of allergens have plant origin, therefore, the aim of this study was to evaluate of the allergenicity effect of *Artemisia deserti* extract. In this research, flowering taps of *A. deserti* were collected from Isfahan around, Iran in September 2012. Flowering taps were extracted using phosphate-buffered saline, PH 7.4 and 80% ethanol. Three concentration of extracts (5, 10 and 15%) were prepared. The experiments were intraperitoneally injection of the extracts 3 times, once every 10 days. In this research the flowering taps allergenicity were detected using subcutaneous, skin scratch, serological and clinical tests. Tests were done on Hartley male Guinea pigs. In treated and control groups the appearance of wheal, their diameter, serum IgE, eosinophilia, neutrophilia were compared. Dates were analysed using ANOVA test in the $p<0.05$. During the skin prick test, the allergenic sensitivity was observed for %15 phosphate-buffered saline extracts, with an average wheal diameter of about 2 cm and for 80% ethanol extracts, with an average wheal diameter of about 3.2 cm. There are significant changes of allergenicity effects between treatment samples and control samples ($p<0.05$). Results of blood smears were seen that, the numbers of eosinophils, neutrophils and amount of IgE were increased in the treated animals with extracts comparing control groups. The results are shown that the higher allergenicity of 80% ethanol extracts as compared with phosphate-buffered saline extracts probably due to the existing of carotenoids at 80% ethanol extracts.

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Poster session – Friday 5th July 2013

A11.91

Comparative study of the *Withania coagolans* root extract and morphine on the spermatogenesis in mice

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Plants have always been a rich source of lead compounds e.g. morphine, cocaine, digitalis, quinine, etc. Therefore, the aim of this study was to comparative study of the *Withania coagolans* root extract and morphine on the spermatogenesis in mice.
In this research, roots of *W. coagolans* were collected from Sistan and Baluchestan around, Iran in June 2012. They were dried under shade and 20 g of flower powder were extracted with 200 mL methanol by Soxhlet extraction for 12 hours. The animals were divided into six groups. Group 1 was injected with saline and tween80 (4:1), group 2 and 3 were injected with extract 100 mg/kg and 200 mg/kg respectively, group 4 was injected with sulphate morphine (5,10, 15 mg/kg) for 6 days, group 5 and 6 were injected with sulphate morphine and extract 100 mg/kg and 200 mg/kg respectively for 6 days. The animals were anaesthetized and the testis tissue was separated and pathological changes were studied.
Treatment with sulphate morphine disturbed spermatogenesis stages, but, treatment with extracts disturbed spermatogenesis stages less than sulphate morphine, also, treatment with sulphate morphine and extract showed fewer disturbances in spermatogonia, spermatid and mature sperm.
Withania coagolans has the antioxidant property that can reduce free radicals-induced oxidative stress. Thus, some of the useful effects of coagolans in this experiment are attributable to the reduction of stress oxidative, also, much of *A coagolans*'s pharmacological activity has been attributed to two main with anolides, with aferin A and with a coagin.

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Poster session – Friday 5th July 2013

A11.92

Male mealworm beetles increase resting metabolic rate under terminal investment

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Harmful parasite infestation can cause energetically costly behavioural and immunological responses which potentially can reduce host fitness and survival. It has often been hypothesized that energetic costs of infection should cause resting metabolic rate (RMR) to increase. The hypothesis of terminal investment states that instead of concentrating resources on an immune defence, individuals should allocate resources in favour of current reproduction, when life expectancy is reduced. In this study, we activated the immune system of *T. molitor* males via insertion of nylon monofilament, conducted female preference tests to estimate attractiveness of male odours and measured a possible change of RMR. The results of this study showed that attractiveness of males coincided with significant down-regulation of their encapsulation response against parasite-like intruder. The activation of the immune system increased RMR only in males with heightened attractiveness of their odours and that later suffered higher mortality rates. The results suggest a link between high RMR and mortality, and also show that an increase in odour attractiveness may be costly in *T. molitor.*

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Poster session – Friday 5th July 2013

A11.93

Slow wave activity in the fish gut? The effect of TTX on gut motility

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Gut motility includes both propagating and non-propagating contractions that are controlled and coordinated by enteric nerves and hormones. However, ultimately the frequency is determined by the slow wave activity originating in interstitial cells of Cajal (ICCs). The ICCs are considered to act as pacemakers and show spontaneous rhythmic depolarizations that spread to the smooth muscles. The distribution and function of ICCs in fish is so far little investigated.
In this study we used *in vivo* video-recordings of the proximal intestine of the shorthorn sculpin (*Myoxocepahalus scorpius*) to study motility patterns and how they are controlled. When nerve activity was blocked using tetrodotoxin (TTX), the pattern changed and became much more regular. The predominating activity was orally propagating ripples (contractions of short duration), usually one to three per minute. This is consistent with non-neurally mediated slow wave activity present in other vertebrates, although the frequency seems to be lower in fish (possibly an effect of lower body temperature). As ICC-like cells can be found also in fish it is most likely that they are responsible for this slow wave activity. Furthermore, segmenting contractions as well as anally propagating contractions of lower frequency and longer duration were few after TTX treatment, indicating the importance of enteric nerves to initiate and maintain peristaltic movements both in fed and fasted fish.

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Poster session – Friday 5th July 2013

A11.94

Effects of 5-hydroxytryptamine on the immunolocalization of CHH (crustacean hyperglycaemic hormone) and branchial chamber Na^+,K^+-ATPase in the prawn, *Palaemon elegans*

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Palaemon elegans is a decapods crustacean that can tolerant a broad range of salinity (5–45 ppt). It is widely occurs and breeds in the coastal zone of the Caspian Sea. The relationship between neurotransmitter 5-HT, neurohormone CHH and the Na^+,K^+-ATPase, key enzyme in ion transport, were investigated in the prawn, *P. elegans* by immunohistochemistry method.
In the control group, a good fluorescence of Na^+,K^+-ATPase observed in the epithelial cells of branchial chamber (in the cells of the inner-side of the branchiostegites and in the epithelial cells of epipodites). A positive but weak fluorescence of the Na^+,K^+-ATPase was also found in lacunae sides of the lamellae cells. In the eyestalk hosts, an intensive fluorescence of CHH was observed in the cells of X-organ. Following injection of 5-HT into the haemolymph of samples, a significant decrease observed in the X-organ CHH fluorescence, whereas a significant increase observed in the Na^+,K^+-ATPase fluorescence in the branchial chamber cells. It is previously reported that CHH and Na^+,K^+-ATPase possess main function in crayfish osmoregulation.
Our result showed that, exogenous 5HT can stimulate CHH releasing from X-organ cells and this hormone can stimulate Na^+,K^+-ATPase intensity in the osmorgulatory organs and finally, they increase this prawn osmorgulatory capacity.

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Poster session – Friday 5th July 2013

A11.95

Interactions between xenobiotic detoxification, anaerobic metabolism and circadian rhythms in three-spined sticklebacks

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Aquatic organisms are increasingly exposed to simultaneous low-oxygen conditions and chemicals originating from anthropogenic sources. The non-steroidal anti-inflammatory drug diclofenac is one of the most common pharmaceuticals found in surface waters, but its physiological effects on fish are poorly known.
Previous studies have indicated that diclofenac can activate the aryl hydrocarbon receptor (AhR) and that there is cross-talk between AhR and the hypoxia inducible factor 1 α (Hif-1α). In this study, three-spined sticklebacks (*Gasterosteus aculeatus*) caught from a fresh water stream in Hamburg, Germany, were subjected to a 24-hour hypoxia or a 13-day diclofenac treatment either separately or simultaneously. Liver samples were collected at three time-points during the day. To assess cross-talk between AhR and Hif-1α the activities of lactate dehydrogenase and cyctochrome P450 1A enzymes and levels of their gene transcripts were measured. Moreover, the levels of core circadian clock components were examined to study the potential of diclofenac and hypoxia to affect the regulation of biological rhythms in fish. The results of the study are to follow.

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Poster session – Friday 5th July 2013

A11.96

When to flee and in which direction: Timing and trajectories of collective anti-predator manoeuvres in schooling fish

Stefano Marras (IAMC – CNR, Italy) and Paolo Domenici (IAMC – CNR, Italy)

Group living is a widespread behaviour, observed in many animal taxa. The collective manoeuvres of gregarious animals reflect remarkable coordination, as is commonly observed in fish schools and bird flocks. In fish, collective behaviour has long been assumed to be generated in a self-organized manner in which individuals within the school appear to move in synchrony like an egalitarian superorganism. This view has changed in more recent years because high-speed video observations on the spatial-temporal organization of schooling fish have shown that their anti-predator manoeuvres are not perfectly synchronous and that collective evading manoeuvres may be affected by the characteristics of the threat (e.g., angle of attack and distance). Here, we provide evidence for a non-random escape order in evading schooling fish under threat, demonstrating that leader-follower rules apply even when life or death is a matter of milliseconds. Startle order is related to the positional preference of individuals in the school. Furthermore, we describe how the pattern of collective escape manoeuvres depends on the distance and the direction of the stimulus.

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Poster session – Friday 5th July 2013

A11.97

Angiotensin II and morpho-functional remodelling of the eel heart

Filippo Garofalo (University of Calabria, Italy), Daniela Amelio (University of Calabria, Italy), Carla Capria (University of Calabria, Italy), Maria C Cerra (University of Calabria, Italy) and Sandra Imbrogno (University of Calabria, Italy)

The octapeptide angiotensin II (Ang II), principal effector of the renin–angiotensin system (RAS), is involved in various biological actions including short-term modulation and long-term adaptations. In the eel, Ang II elicits a short-term cardio-modulatory effect. However, information regarding the influence of Ang II on cardiac remodelling is lacking. To fulfil this gap, we used freshwater eels (*Anguilla anguilla*) intraperitoneally injected for 30 days with Ang II (0.4 or 1.2 nmol g BW^{-1}) or with Ang II (1.2 nmol g BW^{-1}) plus the AT2 receptor antagonist CGP42112.
Using an *in vitro* working heart preparation, cardiac performance was evaluated under loading (i.e. preload and afterload) challenges hearts of all groups showed similar Frank–Starling responses. However, in response to afterload increases, stroke volume rapidly decreased in control hearts, while it was better maintained in Ang II-treated counterparts. These effects were abolished by an antagonist of the AT_2 receptor, whose cardiac expression was revealed by western blotting analysis. We also found by immunolocalization and immunoblotting that Ang II influences both expression and localization of molecules involved in cell growth and apoptosis, such as c-kit, apoptosis repressor with CARD domain (ARC), heat shock protein 90 (Hsp90), and endothelial nitric oxide synthase '(eNOS)-like' isoform.
These results point to a role of Ang II in eel heart remodelling, providing new insights regarding the modulation of cardiac plasticity in fish.

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Poster session – Friday 5th July 2013

A11.98

Hissing calls improve survival in incubating female great tits (*Parus major*)

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Nest predation is the most important selective pressure shaping nest-site selection and nest defence behaviour. In this study we tested whether giving hissing calls may improve survival of incubating female great tits (*Parus major*). We found that 66% of incubating females give hissing calls and 33% of females do not give them while attacked in their nest box. The repeatability of the number of hissing calls given was high as was the latency to give the call. Predators attacked hissing and non-hissing females equally often. However, survival of incubating females was different in that hissing females survived significantly better than silent females. We observed responses of feral cats against playbacks of hissing call during their attacks to nest boxes. It was found that hissing calls slow down the attacks, thus, giving incubating females an opportunity of escape. The ability of giving hissing calls may be related to personality type of incubating female great tits, which needs to be tested experimentally.

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Poster session – Friday 5th July 2013

A11.99

The effects of thermal changes on the nitric oxide-dependent modulation of the Frank–Starling mechanism: The eel heart as a paradigm

Daniela Amelio (University of Calabria, Italy), Filippo Garofalo (University of Calabria, Italy), Carla Capria (University of Calabria, Italy), Bruno Tota (University of Calabria, Italy) and Sandra Imbrogno (University of Calabria, Italy)

A fundamental property of vertebrate myocardium is the Frank–Starling mechanism which allows, when the end-diastolic volume increases, that the consequent stretch of the myocardial fibres generates a more forceful contraction. In the eel (*Anguilla anguilla*) heart, nitric oxide (NO) exerts a direct myocardial relaxant effect, increasing the sensitivity of the Frank–Starling response (Garofalo et al., 2009). Using isolated working heart preparations, this study examined the relationship between NO modulation of Frank–Starling response and temperature challenges in the eel.

The results showed that, while in long-term acclimated fish (spring animals perfused at 20°C and winter animals perfused at 10°C) the inhibition of NO production by L-N5 (1-iminoethyl)ornithine (L-NIO) significantly reduced the Frank–Starling response, under thermal shock conditions (spring animals perfused at 10 or 15°C and winter animals perfused at 15 or 20°C) L-NIO treatment resulted without effect. Western blotting analysis revealed a decrease of peNOS and pAkt expressions in samples subjected to thermal shock. Moreover, an increase in Hsp90 protein levels was observed under heat thermal stress.

These data suggest that in fish the NO synthase/NO-dependent modulation of the Frank–Starling mechanism is sensitive to thermal stress.

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Poster session – Friday 5th July 2013

A11.100

A morpho-functional study of *Carassius auratus* L. heart

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This study provides a structural and functional characterization of *Carassius auratus* L. heart. Besides to the classical four chambers, i.e. sinus venosus, atrium, ventricle, bulbus, we described two distinct structures corresponding to the atrioventricular (AV) region and the conus arteriosus. The atrium is very large and highly trabeculated; the ventricle shows an outer compacta, vascularized by coronary vessels, and an innerspongiosa; the bulbus wall is characterized by a high elastin/collagen ratio, which makes it extremely compliant. Immunolocalization revealed a strong expression of activated 'eNOS-like' isoforms both at coronary endothelium and, to a lesser extent, in the myocardiocytes and the endocardial endothelium (EE). The structural design of the heart appears to comply with its mechanical function. Using an *in vitro* working heart preparation, cardiac performance was evaluated at different filling and afterload pressures. The hearts were very sensitive to filling pressure increases. Maximum stroke volume (SV=1.08±0.09 mL/kg body mass) was obtained with an input pressure of 0.4 kPa. The heart was not able to sustain afterload increases, values higher than 1.5 kPa impairing its performance. These morpho-functional features are consistent with a volume pump mechanical performance.

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Poster session – Friday 5th July 2013

A11.101

The NOS/NO system in cardiac and skeletal muscle remodelling of the lungfish *Protopterus annectens*: Switch from aquatic to aestivating conditions

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During aestivation, characterized by prolonged torpor, metabolic depression and biochemical and morpho-functional readjustments, the lungfish heart continues to pump, while the skeletal muscle stops to function but can immediately contract during arousal. The mechanisms of rearrangement occurring in myotomal and myocardial muscles during aestivation and arousal are unknown. Nitric oxide (NO), for its universal role in cardio-circulatory and muscle homeostasis, could be involved in coordinating these stress-induced adaptations. Western blotting and immunofluorescence on cardiac and skeletal muscles of *Protopterus annectens* (freshwater, six months of aestivation and six days after arousal) showed that expression, localization and activity of the endothelial-like nitric oxide synthase (eNOS) isoform and its partners Akt and Hsp-90 are tissue-specifically modulated. During aestivation, phospho-eNOS/eNOS and phospho-Akt/Akt ratios increased in the heart but decreased in the skeletal muscle. Hsp-90 increased in both muscle types during aestivation. TUNEL assay revealed that increased apoptosis occurred in the skeletal muscle of aestivating lungfish, but the myocardial apoptotic rate of the aestivating lungfish remained unchanged as compared with the freshwater control. Consistent with the preserved cardiac activity during aestivation, the expression of apoptosis repressor (ARC) remained unchanged in the heart of aestivating and aroused fish as compared with the freshwater. Contrarily, ARC expression was reduced in the skeletal muscle of aestivating lungfish. Our data indicate that changes in the eNOS/NO system and cell turnover are implicated in the morpho-functional readjustments occurring in lungfish cardiac and skeletal muscle during the switch from freshwater to aestivation, and between the maintenance and arousal phases of aestivation

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Poster session – Friday 5th July 2013

A11.102

Performance curves of enzyme activity in seven closely-related species of Gymnophthalmidae lizards from the Brazilian Caatinga

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Gymnophthalmid lizards from Brazilian Caatingas occupy diverse microhabitats and exhibit remarkable morphological and behavioural variation. A derived lineage of fossorial endemic species is characterized by a snake-like morphology, where undulation is the major locomotor mode and most species are nocturnal. These patterns diverge from those of epigeal species, which are diurnal and exhibit a lizard-like morphology, being either widely distributed across the Caatinga or restricted to relictual forests. This scenario likely evolved in association with changes in physiological aspects, especially those related to muscle physiology, which might correlate with locomotion and behaviour in specific ecological settings. We measured enzyme activity at five temperatures to determine the thermal sensitivity of citrate synthase (CS) and lactate dehydrogenase (LDH) in trunk muscles and brain of seven gymnophthalmid species. Maximum enzyme activity and Q_{10} values were analysed by ANOVAs, and Q10 values were compared between CS and LDH using student's t-test. Results for CS separate species in two groups. One group, which includes the two diurnal lizard-like species from relictual forests, has lower Q_{10} values and enzyme activity maximized at lower temperatures. The other group includes the remaining diurnal and nocturnal species (snake-like and lizard-like morphologies); their enzyme activities reach maximum at higher temperatures and Q_{10} values are higher. In contrast, LDH was less thermal-sensitive, and only punctual differences between species were observed. Results for CS activity suggest that habitat use might explain variation in aerobic pathways, as enzymes of species occupying thermally mild environments perform better at lower temperatures and seem less thermal-sensitive.

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Poster session – Friday 5th July 2013

A11.103

Aerobic metabolism in the short-live *Nothobranchius furzeri*: Influence of temperature on ageing individuals

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Nothobranchius furzeri is an annual species with an extremely rapid life cycle of about six months, and is therefore a relevant model to study the impact of aging on the integrity of key biological functions. In addition to intrinsic factors, environmental parameters are known to influence fish performance (e.g. growth, reproduction, metabolism, locomotion...). The present study investigates the combined effects of ageing and temperature, and more particularly if temperature provokes a slowing down of the senescent-dependant decrease in performance, and thereby, changes in the fitness of *N. furzeri*. The objective of our study was to highlight aerobic metabolic changes in juvenile, adult and senescent *N. furzeri* acclimated at two temperature, 26°C (optimal temperature) and 22°C. In order to determinate standard (SMR) and maximal (MMR) metabolic rates, oxygen consumption was measured by intermittent flow respirometry. Fish were individually tested in size-adapted respirometer chambers during 2 days. Oxygen consumption was continuously recorded in two main activity statuses: (1) individuals were stressed by chasing them until fatigue to estimate the MMR and then (2) they were left undisturbed to measure SMR.
Results will be discussed through a comparison between the metabolism at the two acclimated-temperatures, and all along the life cycle of *N. furzeri*.

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Poster session – Friday 5th July 2013

A11.104

Cardiac function of *Nothobranchius furzeri*, a vertebrate with an extremely short lifespan, acclimated to different temperatures

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*Nothobranchius furzeri i*s an annual fish with an extremely rapid life cycle of six months. The objective of our study was therefore to follow changes in cardiac performances all along the lifespan. Ageing heart undergoes structural modifications associated with decline in cardiac performances and increase in cardiac disease. As cardiac contractility is profoundly influenced by temperature in fish, we tested on isolated cardiomyocytes, the impact of acclimation temperature (26°C, the optimal temperature, and 22°C) on the ageing process of heart. We examined if ageing was associated with a hypertrophy of the cardimoycytes and thereby an increase of sarcoplasmic reticulum (SR) Ca^{2+} cycling and possibly changes in adrenergic regulation. Morphological and functional changes were followed using immunostaining (size, membrane, ryanodine receptor), calcium imaging (Fluo-A/AM) and pharmacological products (hyperpotassic solution, caffeine and epinephrine). Results will be discussed with a structural and functional comparison of cardiac cell between the two temperatures and during the aging of this species.

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Poster session – Friday 5th July 2013

A11.105

The blood cells changes of *Lithobates catesbeianus* after exposure to cadmium

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Frequently employed to monitor water pollution, the assessment of the biological responses of aquatic vertebrate species provides significant information on bioavailability and actual concentration levels of several pollutants. The current study investigated the changes in some circulating blood cell parameters of *Lithobates catesbeianus* tadpoles after acute (48 hours) and chronic (16 days) exposure to Cd (1 $\mu g.L^{-1}$) using the haemoglobin quantification, erythrocytes and leukocytes counting. Tadpoles species have differential sensitivity to the metal exposure. Haemoglobin levels increased significantly (22%) after 48 hours' exposure compared to the control ($p<0.05$) and in mean erythrocyte (55%). After 16 days of exposure, the mean values of erythrocytes increased by 73% compared to the control ($p<0.05$) as well as the MCH (mean corpuscular haemoglobin) increased (30%). There was an increase of lymphocytes (27%) and a decrease of monocytes, basophils and eosinophils (13%, 7% and 9%, respectively) after 48 hours' exposure compared to control. After 16 days, the neutrophils decrease and the monocytes increased (7% and 10% respectively) compared to control.
The general the nature of responses to cadmium stress emphasizes the role of peripheral blood of tadpoles as a sensitive indicator regarding contamination in aquatic environments. However it was not found significant changes in the leukocytes level between 48 hours and 16 days exposure.
Financial support: FAPESP.

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Poster session – Friday 5th July 2013

A11.106

Investigating non-invasive measures of stress in ornamental fish

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The transport of ornamental marine fish presents potential stressors due to confinement and deteriorating water quality, which to date have been the subject of limited research. Common clownfish, *Amphiprion ocellaris* (Cuvier 1830), were subjected to simulated transport to establish how stressed animals were at different stages of transport (from initial capture and handling to 72 hours transport time), and to investigate the efficacy of non-invasive measures of stress. Additionally, water quality measurements were taken from the transport water at key sampling points.

The results suggest that capture, handling and transport are stressful for clownfish, although the stress response appears to plateau at around 48 hours, possibly due to negative feedback from cortisol. Water-borne cortisol measurement of stress were validated against plasma and whole body measures and thus provide an alternative to more invasive methods of sampling, although the challenges of measuring cortisol in salt water are discussed. Water quality measurements reveal that fish are temporarily subjected to relatively high and toxic concentrations of ammonia (correlating to body mass and transport time), which may have long-term effects on the health of the animals, although handling and confinement appear to be highly significant in eliciting the stress response since improving water quality did not reduce water cortisol concentrations after 24 hours. Thus, transport of ornamental fish for over 24 hours does result in significant stress and lengthy transport times should be avoided.

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Poster session – Friday 5th July 2013

A11.107

Can pansteatitis in the Nile crocodile be linked to feeding habits and bioaccumulation of Al and Fe in fish?

Paul J Oberholster (CSIR: Natural Resources and the Environment, South Africa) and Anna-Maria Botha (Stellenbosch University, South Africa)

Incidents of Nile crocodile (*Crocodylus niloticus*) and serrated hinged terrapin (*Pelusios sinuatus*) mortalities in Lake Loskop, South Africa during the past five years have resulted in a decline in the crocodile population from 80 animals to a total of six, and coincided with incidents of fish die-off in Lake Loskop. The crocodile and terrapin mortalities in Lake Loskop were ascribed to pansteatitis (hardening of body fat, changes in body fat colour from white to yellow), causing stiffness in the crocodiles and resulting in their death appears to be associated with the intake of rancid fish fat after a fish die-off. However, to date no unequivocal answer can be formulated on the precise cause(s) of pansteatitis or the die-off of crocodiles in Lake Loskop.

In the study we report on the spatial heterogeneity between different biota sampled in the lake and the possible association between bioaccumulation of Al and Fe in the food chain and occurrence of fish yellow body fat. As well as, the possible association of the latter with the occurrence of pansteatitis at higher levels in the food chain.

From the study it was evident that high concentrations of Al and Fe detected in the yellow body fat of the studied fish species may be related to the feeding habits of these species. It is proposed that their phytoplankton diet could be a contributing factor in the bioaccumulation of Al and Fe in the food chain and the possible development of pansteatitis in predators at higher trophic levels.

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Poster session – Friday 5th July 2013

A11.108

Assessing the importance of marine-derived nutrients to a freshwater harbour seal (*Phoca vitulina*) population through stable isotope analysis

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Iliamna Lake, Alaska is home to a unique population of freshwater harbour seals, and understanding if the lake seals are isolated from the nearby marine population in Bristol Bay. In the absence of data on individual movements, we collected tissues from seals harvested by Alaskan native hunters in spring and summer, and analysed the samples for δ^{13} C and δ^{15} N to assess reliance on marine *versus* freshwater prey resources. Isotope values from along the full whisker length were used to assess seasonal shifts in diet, while values were compared between muscle, kidney, liver, blubber and heart tissues to assess differences in fractionation rates and/or turnover times. In two-thirds of the samples, there was significant variation in δ^{13} C and δ^{15} N along the length of the whisker, suggesting that seals exploit the seasonal pulse of spawning salmon during summer months; limited variation in the remaining seals suggested they foraged on lacustrine species year-round. Overall, δ^{15} N values did not vary significantly within individuals or among tissues suggesting that all soft-tissues have similar turnover times and fractionation rates. In contrast, significant variation in δ^{13} C values both between individuals and among tissue types remained unexplained even after controlling for variation in tissue lipid content. Findings suggest that there are diverse foraging strategies within the lake, and highlight challenges to using isotope ratios to discriminate among foraging strategies in a freshwater ecosystem into which there is significant marine derived nutrient deposition.

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Poster session – Friday 5th July 2013

A11.109

Effects of environmental estrogens on mitochondrial DNA heteroplasmy

Alicia Chivers (Portland State University, United States), Irvine Schultz (PNNL-MSL, United States), Kim Brown (Portland State University, United States) and Josh Faber-Hammond (Portland State University, United States)

Environmental toxicants are ubiquitous throughout the environment as a result of human activity. Among these toxicants, environmental estrogens are a category of particular concern due to their environmental prevalence and potency in altering reproductive traits. While many studies have addressed the detrimental effects of environmental estrogens on both aquatic and terrestrial organisms, few have analysed the potential for these compounds to alter mitochondrial genomic structure. Mitochondria are the primary energy-generating system for all eukaryotic life, supporting all aspects of development, metabolism and growth. Each cell within the body contains many mitochondria which in turn contain multiple copies of their own DNA genome, mitochondrial DNA (mtDNA). Mutations in mtDNA are responsible for a wide range of human diseases such as metabolic syndromes, cancers and obesity. Many mitochondrial diseases are characterized by increased levels of heteroplasmy, multiple mitochondrial DNA haplotypes within an individual. Increased heteroplasmy alters normal mitochondrial function and influences disease initiation and progression. Despite our knowledge about the relationship of heteroplasmy and disease, we still do not have a complete grasp of the mechanisms of heteroplasmy induction. One underexplored potential source is the continuous exposure to environmental toxicants that we experience every day. Here we report our analysis of mitochondrial heteroplasmy on rainbow trout (*Oncorhynchus mykiss*) exposed for 50 days to either 17α-ethyunylestradiol (EE2), EE2 + tamoxifen, or methanol (control). Data analysis will reveal the relationships of the various exposures to differences in mitochondrial heteroplasmy to determine whether EE2 alters mitochondrial genomic structure.

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Poster session – Friday 5th July 2013

A11.110
Does ethinylestradiol enhance zebrafish ovary development? A stereological study

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Endocrine disrupting chemicals (EDC), are widespread through the aquatic ecosystems and are known to adversely affect the development and reproduction of fish. Zebrafish (*Danio rerio*) has long been used as a model in toxicological studies, regarding the effects of different classes of environmental contaminants, such as xenoestrogens. To evaluate the effect of 17α-ethinylestradiol (EE2), a potent synthetic estrogen, on gonad development, zebrafish were exposed to 4 ng/L of EE2, from one hour to 60 days post-fertilization. Fish (n=10) were collected and processed for histological observation. A quantitative (stereological) analysis, with systematic sampling, was made in the female gonads, and both the relative and absolute volumes of the oocytes stages were estimated. The exposure to EE2 promoted the female zebrafish growth and enhanced gonad development, with an increase, up to 46%, of gonad absolute volumes in exposed fish. This increase was mainly due to a rise in the relative volumes of the advanced maturation stages, such as cortical alveoli, vitellogenic and mature oocytes, while the volumetric density of primary oocyte significantly decreased. In conclusion, the EE2 stimulus induced changes in ovary structural compartments, with increased trends for later oocytes stages.
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Poster session – Friday 5th July 2013

A11.111
Can ISSR and RAPD markers allow us to identify zebrafish sex prior to gonad differentiation?

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How sex is determined in zebrafish (*Danio rerio*) is a long-standing question. Zebrafish is an undifferentiated gonochoristic, with all individuals initially developing an immature ovary before the final differentiation to ovary or testis. Also, the XY and ZW sex-determination systems and morphological differences in the chromosomes are absent in this species; being the mechanisms affecting the sex determination and differentiation still poorly understood and the identification of the sex impossible before the gonad differentiation. Thus, this study aimed to identify genetic differences between male and female using two different molecular markers: ISSRs (Inter Simple Sequence Repeats) and RAPDs (Random Amplified Polymorphic DNA). Two bulks were constructed, with DNA from 16 females and 16 males. From the 100 ISSR primers tested, 62 amplified DNA fragments, within these only 2 presented specific amplification in females and 3 in males' bulks. These differences were not confirmed in individual samples analysis. From the 280 RAPD primers tested, 273 amplified DNA fragments, within these 5 amplified specific fragments in the females' bulk and 9 in the males' bulk. The individual samples analysis showed the amplification of a specific DNA fragment in the majority of females and its absence in the male samples. The results suggest that differences among the zebrafish DNA may allow the sexual identification of individuals before the gonad differentiation.
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A11.112
Starfish vision and visually guided behaviours

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Most known starfish species possess a compound eye at the tip of each arm, which, except for the lack of true optics, resembles arthropod compound eye. Despite that these compound eyes have been known for about two centuries, no visually guided behaviour has ever be directly associated with their presence. There are indications that they are involved in negative phototaxis but this may also be governed by extraoccular photoreceptors. Here we show that the eyes of the coral-reef-associated starfish *Linckia laevigata* are slow and colour blind. The eyes are capable of true image formation although with low spatial resolution. Further, our behavioural experiments reveal that only specimens with intact eyes can navigate back to their reef habitat when displaced, demonstrating that this is a visually guided behaviour. This is the first report of a function of starfish compound eyes. We also show that the spectral sensitivity optimizes the contrast between the reef and the open ocean. Our results provide an example of an eye supporting only low resolution vision, which is believed to be an essential stage in eye evolution, preceding the high resolution vision required for detecting prey, predators and conspecifics.

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