A1 – GENERAL BIOMECHANICS

A1.1
Hydrodynamic analysis of suspension feeding in extinct and extant crinoids (Echinodermata: Crinoidea)

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Crinoids belong to the phylum Echinodermata, which today also includes the echinoids, asteroids, ophiuroids and holothuroids. Based on their long and complete fossil record, their great diversity and their passive suspension feeding strategy, crinoids represent ideal objects to study effective biological filter mechanisms.

Fossil representatives can differ strongly in their morphology from recent forms and thus alternative feeding positions can be assumed than those effective today. Recent stalked crinoids open their arms in a parabolic filtration fan, creating a three-dimensional filter to capture nutritive particles that are in the water. Some of the fossil representatives were possibly not as flexible, and thus not able to bend their arms to such an extent. Furthermore, recent stalked forms are restricted to deeper water settings with relatively constant flow conditions, while fossil crinoids mainly lived in shallow water habitats, where flow velocities and directions changed more rapidly.

The interdisciplinary project presented analyses the filter-feeding mechanisms of recent and fossil crinoids using flume experiments (particle image velocimetry) as well as modern computational approaches (computational fluid dynamics). In a first attempt, two crinoids are analysed: *Encrinus liliiformis* (Middle Triassic, 243–228million years ago) with 10 simple arms, and recent *Hyocrinus* sp. with five unbranching arms. For *Encrinus*, analyses reveal that – based on the general crown shape – a backward current is generated leading food particles back into the crown. For the recent crinoid *Hyocrinus*, further analyses are needed, including more detailed morphological structures that were found to be essential for the circulation pattern around the animal.

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11:00 Friday 29th June 2012

A1.2
Biomechanics of prey capture in the Chinese giant salamander and what it might tell us about early tetrapod evolution

Egon Heiss (University of Antwerp, Belgium and University of Vienna, Austria) and Sam Van Wassenbergh (University of Antwerp, Belgium)

An important step in furthering our understanding of tetrapod evolution is to identify how early amphibians managed to capture prey, after substantial changes in the anatomy of their feeding system during the fish–tetrapod transition. Similar to their sarcopterygian ancestors, the first amphibians were large aquatic predators that, in many aspects, resembled today's basal amphibian species of Asian giant salamanders. Consequently, functional morphological insights into the feeding strategy employed by these 'living fossil' amphibians might explain how profound modifications of the cranial architecture could have occurred without a major loss in prey-capture performance. Here, we show that the broad-skulled Chinese giant salamander, *Andrias davidianus*, uses a novel, exclusively jaw-powered suction mechanism to capture prey. Analysis of the skeletal morphology, high-speed video recordings and computational fluid dynamics simulations show how a fast separation of its large upper and lower jaws creates explosive, high flow velocity suction, while the viscerocranial elements mainly serve to accommodate the engulfed water volume. A shift in function of the viscerocranium from its primitive, obligatory active role in powering suction to a more passive, supporting role mgiht have released the biomechanical constraints on the evolution of the hyobranchial system within the early tetrapod lineage, allowing development of a muscular tongue.

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11:15 Friday 29th June 2012

A1.3
Biting force as a key factor for ecological diversification in South American caviomorph rodents

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Within mammals, the South American caviomorph rodents became one of the most diverse clades considering their occupied habitats, diets, locomotor modes and body sizes. This might have been partly achieved by diversifying their feeding system and its ecological commitment, e.g. chisel-tooth digging. Thus, both powerful jaw musculature and biting force, exerted at the tip of the incisors, might have allowed these rodent species to differentiate in their environmental use.

The *in vivo* forces of specimens of three southern South American caviomorph species – the chinchilla (*Chinchilla lanigera*, which is social and ground dwelling), the degu (*Octodon degus*, which is social and fossorial) and the sand dune tuco-tuco (*Ctenomys australis*, which is solitary and subterranean/claw and chisel-tooth digger) – were measured. Estimations of biting forces were also achieved by measuring the dissected jaw muscles, and their in- and out-levers, considering biting forces as reaction forces to food particles acting at different angles on the incisors' tips (from 30º higher to 30º lower than the maximum out-lever at the incisors' tips).

We found that estimated biting forces were statistically higher than *in vivo* forces. Since the species of tuco-tuco studied displayed the highest *in vivo* relative values and the chinchilla the lowest ones, we assumed that the biting force would mainly be associated to the fossorial status and aggressive territorial behaviour, which seems common in tuco-tucos. Thus, our functional approach would complement the morphological assessments carried out so far on caviomorph rodents' skull and jaw structure and muscles.

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11:30 Friday 29th June 2012

A1.4
The muscle, its force, its heat and its structure: Just elasticities in series?

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Recently, it was demonstrated that the Hill relation can be derived from a very simple, macroscopic structural arrangement of mechanical elements. Compared to the force–velocity relation, the corresponding heat rate relation seems to contain even more subtle information about muscle structure. Based on this, we present a revised muscle model that can fit both relations perfectly, using not more than six parameters. For this, a much lower dissipation coefficient, when compared to that in parallel to the source of active force generation, is predicted in series. The main result is that the model explains both relations at once by the interaction of:

- an active force generator (active element);
- energy dissipation in parallel to the AE (parallel damping element);
- some partitioning between presumably elastic (serial element) and dissipative forces (serial damping element) in series to the active element.

As a consequence, the assumption that any series elastic element is at constant length in isotonic contractions should be reconsidered.

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11:45 Friday 29th June 2012

A1.5
Does hibernation affect skeletal muscle performance in 13-lined ground squirrels?

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Many animals hibernate to avoid seasonally low temperatures and scarcity of food. During hibernation, ground squirrels undergo a series of torpor bouts, each lasting more than a week. Previous studies on the effects of hibernation on skeletal muscle have demonstrated that such natural models of muscle disuse can cause significant skeletal muscle atrophy, but limited or no changes in mechanical performance. Work loop analysis of skeletal muscle performance in mammalian hibernators, however, has only previously been undertaken on hamsters, which undergo short, daily torpor bouts.

In the present study our aim was to assess the effects of three months of hibernation on mechanical performance, glycogen content and total antioxidant capacity of soleus skeletal muscle and mitochondrial oxidative capacity of hind limb skeletal muscle in 13-lined ground squirrels. There was no significant difference in peak work loop soleus muscle power output between winter (torpid) and active summer (control) animals. Soleus muscle fatigue resistance was significantly lower, however, in torpid than summer animals. Total antioxidant capacity of the soleus muscle was significantly higher (approximately 2.5 times higher) in torpid than summer animals, suggesting one potential mechanism for maintenance of acute muscle performance. There was no significant difference in soleus muscle glycogen content between summer and torpid animals, but state 3 mitochondrial respiration was significantly suppressed (by about 60%) in hind limb skeletal muscle from torpid animals compared with summer controls. This suggests a potential mechanism that, although useful for metabolic rate suppression, might reduce skeletal muscle fatigue resistance during hibernation.

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12:00 Friday 29th June 2012

A1.6
Economical muscle function during running: Do tendon springs save energy?

Natalie Holt (Harvard University, Massachusetts, USA), Thomas J. Roberts (Brown University, Rhode Island, USA) and Graham N. Askew (University of Leeds, UK)

The long distal tendons of cursorial animals are thought to have evolved to reduce the metabolic cost of running. During level running, force must be produced to support bodyweight while the cyclical fluctuations of the mechanical energy of the body occurring over the course of a step are accommodated. It is assumed that this can be achieved more economically if muscle generates force isometrically and tendon stretches and recoils than if muscle produces force while stretching and shortening, as muscle actively shortening and doing work is metabolically expensive. However, tendon stretch and recoil also reduces the metabolically-inexpensive force production during stretch. Thus, to evaluate the metabolic benefits of tendon, muscle ergometry and myothermic techniques were used to determine the cost of producing force in frog iliofibularis muscles contracting isometrically and undergoing active stretch–shorten cycles; conditions that simulated muscle action with and without effective tendon energy storage and recovery respectively.

If isometric muscle contraction with tendon stretch and recoil represents the most economical way for muscle to function during level running, the cost of force production would be lower in isometric than stretch–shorten cycles. However, we found that there was no significant difference (p=0.15) in the cost of force production in isometric (20.1 ±3.6 mJ N^{-1} s^{-1}) and stretch–shorten cycles (15.1 ±3.6 mJ N^{-1} s^{-1}). This suggests that tendons do not provide some of the energetic benefits previously assumed and that their ability to reduce work done by muscle might not have been the driving force behind their evolution.

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12:15 Friday 29th June 2012

A1.7
Determinants of passive force production in muscle during locomotion

Thomas J Roberts (Brown University, Rhode Island, USA) and Nicolai Konow (Brown University, Rhode Island, USA)

The spring-like properties of passive muscle hold the potential to provide an economical means of powering limb movement, as parallel elastic elements can store and return energy with virtually no metabolic cost. Passive muscle elasticity is less resilient than tendon, however, and is presumably only engaged near the limits of a joint's range of motion.

We investigated the role of passive muscle force in the turkey lateral gastrocnemius during running, using strain gauges mounted on bony tendon to measure muscle force and sonomicrometry to measure fascicle length. Passive tension in the gastrocnemius has the potential to engage during swing phase, when ankle flexion and knee extension stretch the muscle to long lengths. Muscle force predicted from the static *in situ* length curve was much lower than the force observed during running, and this observation, taken alone, would suggest that passive tension is unimportant but we find that passive force at a given muscle length is significantly greater:

- if the muscle undergoes rapid lengthening; and
- if the passive stretch is preceded by an active contraction.

The former is explained by the viscoelastic nature of passive muscle and the latter by the tendency of active muscle contractions to transiently enhance passive force. With the effects of stretch velocity and force enhancement included, the calculated contribution of passive muscle tension is 40–60% of the swing phase force. Passive force production in skeletal muscle might therefore be more flexible and more important in locomotion than is generally appreciated.

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12:30 Friday 29th June 2012

A1.8
Evidence for a muscle power limitation to speed: Scaling of muscle function and hydrodynamics constrains maximum power in swimming frogs

Christofer J. Clemente (Harvard University, Massachusetts, USA) and Christopher Richards (Harvard University, Massachusetts, USA)

Studies of the muscle force–velocity relationship and its derived 'n-shaped' power–velocity curve offer important insights into muscular limits to swimming performance. Although power is maximal at one-third V_{max}, geometric scaling of muscle force coupled with fluid drag force implies that this optimal velocity for power cannot be maintained across the natural body size range. Instead, muscle velocity can decrease with increasing body size, conferring a similar n-shaped power curve with body size.

To test this we examined speed and muscle function for the aquatic frog, *Xenopus laevis*. Across two orders of magnitude of body mass, speed showed an n-shaped relationship, peaking at 47.35 g, while ankle angular velocity decreased linearly. Further, *in vitro* scaling of isometric muscle force and V_{max} of the ankle extensor plantaris longus not only predicted the observed scaling of ankle angular velocity, but the optimal body mass for muscle power output (47.27 g) also coincided with that of swimming speed. Thus, current findings suggest that in drag-based aquatic systems, muscle–environment interactions vary with body size, limiting muscle's potential to produce power and swimming speed.

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12:45 Friday 29th June 2012

A1.9
Using numerical and robotic approaches to understand muscle–tendon power amplification during swimming

Christopher T. Richards (Harvard University, Massachusetts, USA), Udit Choudhury (Harvard University, Massachusetts, USA) and Gregory Sawicki (North Carolina State University and University of North Carolina Chapel Hill, California, USA)

In some animals, the mechanical power required to jump exceeds the theoretical limits of muscle power. To achieve this, a muscle stores elastic energy in the tendon before it is released rapidly, producing 'power amplification' as tendon recoil assists the muscle to accelerate the load.

Using a Hill-type mathematical model of a muscle–tendon (MT), we determined whether power amplification can occur against a fluid dynamic load. Additionally, we used a hybrid computational-experimental approach using a Hill-type MT model to actuate a robotic limb through water. The MT was modelled with contractile element and series elastic element properties measured from frogs. We simulated muscle contractions with limb masses ranging from 0.3 to 3.0 to 30.0 g, foot-fin areas from 0.005 to 0.05 to 0.5 to 5.0 to 50.0 cm^2 and effective mechanical advantage (in-lever/out-lever) of 0.025 to 0.05 to 0.1.

Certain conditions produced power amplification where the MT produced power ~19% greater than the limit for muscle alone. Overall, power output was most sensitive to changes in effective mechanical advantage. Power amplification was only observed to occur if peak inertial force was at least 1.5-fold greater than peak hydrodynamic drag force.

Underlying these interactions, simulation and experimental data predict that variation in limb morphology can tune contractile element power, stored series elastic element energy and the timing of elastic recoil to enable MT power outputs over a range of locomotor environments.

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13:00 Friday 29th June 2012

A1.10
Tails induce transitions in lizards: Righting in mid-air using appendage inertia

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Unlike the falling cat, lizards right in mid-air by swinging their large tails in one direction causing the body to rotate in the other. To investigate the mechanics, we conducted righting experiments on Flat-tailed house geckos (*Hemidactylus platyurus*) and Green anoles (*Anolis carolinensis*) of similar body size, but differing tail length. We used a 3D computational model to compare the effects of variation in tail length and orientation (Jusufi, Kawano, Libby, Full, 2010). Using the morphological parameters of geckos, we found that air-righting in the model matched the degree of roll and yaw directly measured in the animals. Greater tail length in anoles allowed swinging at shallow tail angles to produce a full reorientation of the body. By using a physical model, a robot named RightingBot, we showed that effective attitude control can be attained with simple movements of an inertial appendage. The maximum drag moment from aerodynamic forces acting on the rapidly rotating tail accounted for <5% of the total moment required, indicating that non-inertial torques are less likely to play a major role. Transitions from a given initial orientation to the desired dorsoventral posture must necessarily precede other aerial behaviours, such as gliding flight and perching. Inertial control could be effective for airborne animals at low speeds or high angles of attack, where lift-based control mechanisms do not suffice to generate significant changes in body orientation. Aerial righting responses appear to be widespread among terrestrial arthropods and vertebrates (Jusufi, Zeng, Full, Dudley, 2011).

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14:00 Friday 29th June 2012

A1.11
Biomechanical analysis of take-off in birds

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Take-off is a crucial component of avian flight but its mechanical aspects are not well understood. To investigate the relative contribution of wings and legs during take-off, we integrated measures of leg and wing forces during take-off and the first three wing beats in zebra finch (*Taeniopygia guttata*, 15 g, N=7) and diamond dove (*Geopelia cuneata*, 50 g, N=3). We measured ground-reaction forces produced by the hind limbs using a perch mounted on a force-plate, whole body and wing kinematics using high-speed video, and aerodynamic forces using particle image velocimetry. When birds were perched, an acceleration peak produced by the legs contributes to 85.0 ±12.0% of the whole body resultant acceleration in finch and 77.0 ±6.1% in dove. At lift-off, coincident with the start of the first down-stroke, the percentage of hind limb contribution to initial flight velocity is 93.6 ±0.6% in finch and 92.1 ±0.4% in dove. In finch, the first wing beat produces 57.9 ±3.4% of the lift created during subsequent wing beats compared to 62.5 ±2.2% in dove. These results underscore the relatively low contribution of the wings to initial take-off. We performed a three-dimensional kinematic analysis of the hind limbs, using XROMM data. This analysis revealed that two successive phases occur when the bird is still on the perch and contribute to the initial acceleration peak. These results will help understand the mechanical demands imposed by take-off and lead to a better understanding of the role of the hind limbs in bird locomotion.

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14:15 Friday 29th June 2012

A1.12
Quantifying perching manoeuvres in the steppe eagle *Aquila nipalensis*

Anna C. Carruthers (Oxford University, UK), Simon W. Walker (Oxford University, UK), Shane P. Windsor (Oxford University, UK) and Graham K. Taylor (Oxford University, UK)

Gliding birds decelerate from relatively fast forwards flight to near-zero forwards velocity when coming in to perch. The fact that they accomplish this within a fraction of a second is spectacular for the onlooker, but also has potential applications for unmanned air vehicles. Here we examine the detailed kinematics of an eagle during gliding perching manoeuvres. Multiple high-speed cameras were used to record sequences with wind speeds measured using an ultrasonic anemometer. An in-house automated shape-carving technique is used to analyse the video data and obtain full three-dimensional reconstructions to provide detailed body, wing and tail kinematics. A principal components analysis is conducted on the aerodynamic and kinematic variables extracted from a reduced number of manually-digitized markers on the wings and tail. The primary variables required for description of perching manoeuvres are then identified.

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14:30 Friday 29th June 2012

A1.13
Intraspecific chasing performance in barn and cliff swallows

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Free flight trajectories and behaviour were recorded from intraspecific chases of wild barn (*Hirundo rustica*) and American cliff swallows (*Petrochelidon pyrrhonota*) via high-speed (100 Hz) stereo videography.

Chases, where one bird follows the other through a series of high-speed turns are particularly revealing of the neurosensory and biomechanical latencies of manoeuvring flight. Latencies ranged from 40 to 90 milliseconds, i.e. approximately one-half to one wing beat in these birds. Furthermore, the exact latency depended on the phase of the wing beat of the chasing bird with respect to the leading bird. However, we did not find evidence for phase locking by the chasing bird.

These preliminary results suggest that biomechanical latencies might be more important than neurosensory latencies in these animals. Unlike data recorded from flying insects, these recordings do not strongly support a 'constant visual bearing' tracking mechanism, suggesting that swallows might be more predictive in their target tracking. Intraspecific chases could also provide insight into flight performance capabilities; maximum flight speeds quantified in the study to date were 11.7 m s^{-1}, maximum accelerations were 36.4 m s^{-2}; and minimum curvature was 0.14 m.

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14:45 Friday 29th June 2012

A1.14
The influence of wing design and flapping frequency on the four-dimensional flow pattern of a model wing at bird scale

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The effect of airfoil design parameters, such as airfoil thickness and camber, are well understood in steady-state aerodynamics. Flow visualizations and computational analyses of flapping insect flight have identified several unsteady effects that contribute substantially to the aerodynamic force generation. In flapping flight, high angles of attack and partly separated flow are common features. It is therefore expected that airfoil design parameters affect unsteady wing aerodynamics differently.

Several studies of wing design using revolving wings at insect scale did not find any noticeable effect on lift and drag coefficients. Similar studies of revolving bird wings at higher Reynolds number, however, identified wings with camber and a sharp leading edge as creating higher forces.

We studied the time-resolved flow field around and behind different flapping wing models in translational flow at a Reynolds number of 22.000 < Re < 26.000, mimicking the slow flight of birds. The flow was mapped in three dimensions over the whole flapping cycle analysing perpendicular stacks of two-dimensional planes using particle image velocimetry. The combined effect of several Strouhal numbers (0.2 < St < 0.4), camber and thickness on flow morphology and on circulation was analysed. The resulting measurements of flow phenomena, circulation and lift estimates will be presented and discussed.

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15:00 Friday 29th June 2012

A1.15
Cost of compensation for wing asymmetries in the hawkmoth *Manduca sexta*

María José Fernández (University of Leeds, UK), Ellis Driver (University of North Carolina at Chapel Hill, North Carolina, USA) and Tyson L. Hedrick (University of North Carolina at Chapel Hill, North Carolina, USA)

Over time, irreparable wing damage in flying insects is an inevitable phenomenon and generally results in wing asymmetries. Despite asymmetrically-damaged wings, flying insects are able to maintain stability. Recently we found that hawkmoths control and maintain stability during hovering through neural modulation of muscle activity, specifically by constantly steering to counteract the torque produced by the wing asymmetry. We therefore expect to see a cost to this constant steering on other aspects of flight performance, such as maximum lift production, where some aerodynamic force will be assigned to flight control in addition to weight support. Moreover, the reduction in wing area and consequent control costs could also affect the energetics of flight.

We quantifyed the cost of flying with damaged wings, specifically with asymmetric and symmetric artificially-reduced wings, by measuring maximum load lifting capabilities, oxygen consumption and carbon dioxide production during hovering flight in hawkmoths (*Manduca sexta*). Both the maximum force and metabolic cost experiments exhibited similar results. In both cases, we found that asymmetric wing reduction substantially reduced maximum flight force and flight efficiency (i.e. N/W). Further reducing wing area by reducing the undamaged wing to symmetry with its previously damaged pair led to no changes in maximum force or metabolic cost. We therefore suggest that there is a significant cost relating to control in addition to the cost of the total reduced wing area seen in our asymmetric and symmetric wing-clipping treatments.

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15:15 Friday 29th June 2012

A1.16
Enigma of pterostigma: An inertial regulator for deformation of flapping wings in dragonflies

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Pterostigma, a thickened and heavy region of the leading edge on insect wing, is believed to stabilize the wing during gliding, yet its role during flapping flight remains unclear. In this study, we examined whether pterostigma would affect span-wise wing deformation (bending) in a dragonfly, *Pantala flavescens*. Being flapped by a shaker at fixed frequency and amplitude, the intact wings had greater deformation than those without pterostigma, and bent asymmetrically between the up- and down-strokes. These results were consistent with the predictions from loss of pterostigma mass, and provided the first evidence that pterostigma can enhance wing deformation by inertial effect.

In living dragonflies, however, the loss of pterostigma did not significantly change wing deformation but lead to increased flapping amplitude, presumably to maintain similar wing deformation. When we normalized bending deformation by flapping amplitude, the effects of pterostigma mass was revealed again. The fact that dragonflies increase flapping amplitudes to compensate for the loss of pterostigma at a possible cost of greater energy expenditure suggests a functional importance of wing deformation.

Recent literature provides evidence that wing deformation can facilitate lift production; therefore, wing deformation enhanced by the inertia of pterostigma might be important to the flight performance of dragonflies.

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15:30 Friday 29th June 2012

A1.17
Formation and evolution of the leading-edge vortex on an insect-like flapping wing throughout a half-stroke

Nathan Phillips (Cranfield University, UK)

Flow field measurements were performed on an insect-like flapping wing to characterize the formation and growth of the leading-edge vortex (LEV), a lift-augmenting flow structure also exploited by delta-wing aircraft. The chosen scale was that relevant to small (~150 mm wingspan) insect-inspired flapping-wing micro air vehicles, which operate in the higher order end of the Reynolds number (ratio of inertial to viscous fluid forces) range in which insects fly.

Experiments were accomplished with a custom-designed, mechanical flapping-wing apparatus (the 'Flapperatus') that mimics insect-like flapping-wing motion, with adjustable kinematics up to a 20 Hz flapping frequency in air. Stereoscopic particle image velocimetry was employed to perform phase-locked pseudo-volumetric flow field measurements at various points throughout a half-stroke (one-half of a flapping cycle). Vortex axis trajectories and characteristics of the LEV were recovered from the measurements to reveal the three-dimensional structure of the LEV.

Results revealed that at this scale throughout a half-stroke the LEV grows in size and develops a strong axial velocity through its core towards the wingtip. The LEV also exhibited signs of vortex breakdown, with a higher Reynolds number effect observed in the LEVs of delta-wing aircraft. Despite the presence of breakdown, however, the LEV was observed to remain attached to the wing surface throughout the entire half-stroke.

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16:15 Friday 29th June 2012

A1.18
From small to large: Efficiency of lift production in six species of hawkmoths

Per Henningsson (University of Oxford, UK) and Bomphrey J. Richard (University of Oxford, UK)

The efficiency of lift production is important for all flying animals – irrespective of phylogeny or ecological niche – because it directly influences the limits of performance. For both fixed-wing vehicles and flapping animals, span efficiency, e_i, can be estimated using quantitative flow diagnostics and fundamental aerodynamic theory. Wings generating lift in the most aerodynamically efficient way do so by deflecting the oncoming airflow uniformly across the span, creating a uniform span-wise downwash velocity distribution. Any deviation from uniformity is associated with an extra cost as induced drag increases. By quantifying how large this deviation is, the increase in drag and the reduction in span efficiency can be calculated. We used high-speed stereo particle image velocimetry with a repetition rate of 1 kHz to capture the near wake from six species of hawkmoths flying tethered in a wind tunnel. The selected species represent a range in wingspan from 40 mm to 110 mm (2.75 times) and in mass from 0.2 g to 1.5 g (7.5 times). From the high spatiotemporal resolution flow fields, we extracted time-resolved downwash distributions behind the animals, calculating instantaneous values of e_i throughout the wing beat cycle as well as multi-wing-beat averages. Here we present how time-varying span efficiency differs between the six moth species and discuss the effect of morphological and kinematic parameters.

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16:30 Friday 29th June 2012

A1.19
Predator *versus* prey: Biomechanics, behaviour and strategy during aerial predation in dragonflies

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Despite extensive research on the ecology and population dynamics of predator–prey systems, our understanding of the physical interaction and the factors that determine the outcome of an encounter remains limited. We performed controlled predation trials and analysed high-speed videos of dragonflies (*Libellula cyanea* and *Sympetrum rubicundulum*) hunting fruit flies (*Drosophila melanogaster*) to understand how flight mechanics and behaviour of predator and prey affect the outcome of these encounters.

The flight performance of fruit flies in our natural, outdoor enclosure differs significantly from previously published accounts of *Drosophila* flight capabilities in the laboratory. The pursuit strategy and capture success of dragonflies is highly dependent on prey behaviour. Fruit flies perform a series of random turns (saccades) during normal cruising flight, but the speed and curvature of these turns varies between individuals. Rather than adopting the most direct route to interception, dragonflies approach their prey from below, optimizing the ability to visually track prey and minimizing the likelihood of being detected. Fruit flies that perform frequent, rapid saccades are harder for dragonflies to approach in this manner, and occasionally detect the approaching predator in time to initiate evasive manoeuvres. Overall, dragonflies are more successful at capturing fruit flies that perform slow, moderate turns during cruising flight. These results highlight the difficulty in fully understanding complex flight behaviours, such as predation and escape, through experiments performed in artificial settings or by eliciting these behaviours with artificial stimuli.

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16:45 Friday 29th June 2012

A1.20
Insect wing flexibility enhances aerodynamic force production

Andrew M. Mountcastle (Harvard University, Massachusetts, USA) and Stacey A. Combes (Harvard University, Massachusetts, USA)

The effect of wing flexibility on aerodynamic force production has emerged as a central question in insect flight research yet physical and computational models have yielded conflicting results on whether wing deformations enhance or diminish flight forces. By experimentally manipulating wing flexibility in live bumblebees, we demonstrate that wing flexibility affects flight performance in a natural behavioural context. Bumblebee wings were artificially stiffened *in vivo* by applying a micro-splint to a single flexible vein joint. Bees with stiffened wings showed a significant reduction in maximum aerodynamic force production and load-lifting capacity. Our results reveal that flexible wing design for passive deformations is a critical determinant of insect flight performance, with important ecological implications.

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17:00 Friday 29th June 2012

A1.21
Time-resolved three-dimensional visualization of the blowfly flight motor

Simon M. Walker (Oxford University, UK), Daniel A. Schwyn (Imperial College London, UK), Rajmund Mokso (Paul Scherrer Institute, Switzerland), Martina Wicklein (Imperial College London, UK), Michael Doube (Max Planck Institute of Molecular Cell Biology and Genetics, Germany), Marco Stampanoni (Paul Scherrer Institute, Switzerland) and Graham K. Taylor (Oxford University, UK)

In flying insects, control of wing kinematics is driven by subtle changes in the conformation of the steering muscles that connect to the intricate wing hinge. These muscles have been characterized in several dipteran species, with most of our knowledge of their function coming from microscopy and electrophysiology. The movements of these muscles have never been directly observed *in vivo*.

We used synchrotron-based high-speed X-ray microtomography to perform non-invasive *in vivo* imaging of the flight motor system in tethered blowflies (*Calliphora vicina*). We simultaneously tracked the wings using stereo high-speed digital cameras, which we used to reconstruct the three-dimensional wing kinematics and to gate the tomograms for different stages of the wing-beat cycle. The high phase contrast between tissue and air enabled the reconstruction of the fly's thorax during a composite wing beat with sub-millisecond temporal resolution and voxels with an edge length of 3 µm.

Virtual dissections of the thorax meant that changes in length and volume of the indirect flight muscles could be measured and the majority of the direct, steering muscles could be identified and visualized throughout the wing beat. The rotation of the insect during the experiment, a requirement for synchrotron-based microtomography, was used to stimulate a strong compensatory roll response in the flies. This produced consistent asymmetries in the steering muscles and the corresponding wing kinematics, providing further insight into how the steering muscles are used to control the kinematic output of the wings.

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17:15 Friday 29th June 2012

A1.22
The galloping dung beetle: A new gait in insects and its consequences for navigation

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In the deserts of south-western Africa, 13 species of flightless dung beetles *Scarabaeus* (Pachysoma) feed on the dry dung of small mammals and dead plant matter. After locating a food source, they dig a burrow and then repeatedly drag small loads of food towards it. While foraging, the plant-feeding species walk in a unique 'galloping' gait that has never been described in any insect before. Like a bounding hare, the beetles propel their body forward by synchronously stepping with both middle legs and then both front legs.

In this study, we describe this unique gait and its evolution, and investigate its consequences for visual navigation. Is this gait more efficient on loose sand? Does it relate to the beetles' herbivorous ecology and the fact that they lift their hind legs when dragging plant detritus to their burrow? What are the consequences for visual homing in these path-integrating central-place foragers? In the galloping species, the forward-facing ventral eyes are drastically reduced compared to the dung-collecting species that walk in the usual insect 'tripod' gait. Could this reflect different navigational strategies?

Future research into this novel gait might provide an interesting new model organism for the study of biomechanics and insect navigation.

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10:25 Saturday 30th June 2012

A1.23
Does locomotion suffer due to enormous mandibles in stag beetles?

Jana Goyens (University of Antwerp, Belgium), Peter Aerts (University of Antwerp, Belgium) and Joris Dirckx (University of Antwerp, Belgium)

Sexual selection in stag beetles has caused the emergence of enormous mandibles in males, which are used as weapons in disputes over females. Other morphological differences between sexes have been found using observations as well as computed tomography-scans, e.g. larger heads, longer limbs and an anteriorly shifted centre of mass in males. The latter adaptations might be related to the enlarged mandibles.

It is likely that the pronounced morphological changes have important functional consequences on ecological functions, such as locomotion. We performed high-speed recordings of the locomotion of *Cyclommatus metallifer* individuals. Similar to many other hexapods, these stag beetles show an alternating tripod gait. Males and females are able to walk at the same relative speed and with the same relative step size. They also bear similar yaw, pitch and roll rotations. Males appear to be able to keep their locomotion pattern similar to that of females by supporting the heavy head with their long anterior limbs. In both sexes, the kinetic and potential energy fluctuate nearly in phase. To travel the same distance, however, the total energy per mass is higher for males. The extremely large mandibles of male stag beetles therefore appear to increase the locomotion cost, but not to drastically limit locomotion performance.

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10:40 Saturday 30th June 2012

A1.24

Fast legged locomotion in the microscale – global kinematics and dynamics in the wood ant *Formica polyctena*

Lars Reinhardt (University of Jena, Germany), Tom Weihmann (University of Jena, Germany) and Reinhard Blickhan (University of Jena, Germany)

The kinematics and dynamics of large arthropods have been extensively studied in the past decades. Thus, for fast-running cockroaches and ghost crabs, rhythmical fluctuations of the centre of mass (CoM) that are equivalent to those of running and trotting vertebrates have been observed. For more than 100-fold smaller animals like ants, few examinations are available. Locomotion dynamics are therefore largely unknown in small-legged arthropods. We measured the foot-fall positions of all legs and the time course of the CoM in worker ants (weight ≈20 mg) of the species *Formica polyctena* in three dimensions with a high-speed video system at 500 Hz. These animals are specialized in fast locomotion across varying substrates by using regularly alternating sets of diagonally adjacent legs (tripods). In our experiments, the specimens ran at a mean speed of 0.10 ms^{-1}, which corresponds to 10 body-lengths per second.

To calculate the position of the CoM, the positions and masses (accuracy: 0.1 mg) of three major body parts (head, thorax and abdomen) had to be determined. They are defined by four anatomic points: mandible tip, cervix, petiolus and posterior gaster tip. During the entire stride period, the position of the CoM was very close to the petiolus. Although the characteristics of lateral fluctuations are very similar to those of much larger cockroaches, in the sagittal plane the CoM reached its highest position during midstance. In contrast to larger arthropods, ants do not use spring-mass dynamics but follow the rules of a simple inverted pendulum.

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10:55 Saturday 30th June 2012

A1.26

Spiders don't run: Fast locomotion in large spiders does not follow inverted-pendulum or spring-mass dynamics

Tom Weihmann (Friedrich Schiller University, Germany)

Spiders are an old but successful predatory group of arthropods. Their locomotor system differs from those of most other arthropods by the lack of extensor muscles in two major leg joints. Though specific functional characteristics can be expected for the locomotion dynamics of spiders, this aspect of movement physiology has scarcely been examined. This study presents extensive analyses of a large dataset of the global kinematics and dynamics of adult female specimens of the large Central American spider *Cupiennius salei*. The experiments covered the entire speed range of straight runs at constant speeds. The analyses revealed specific characteristics of velocity-dependent changes in single leg movements as well as in the translational and rotational degrees of freedom of the centre of mass and the body axis, respectively. Thus, in contrast to many other fast-moving arthropods, *C. salei* seem to avoid fluctuations in the centre of mass during fast locomotion and do not exploit spring-mass dynamics. Consequently, aerial phases could not be observed here. This behaviour is probably a consequence of optimizing energy expenditure with regard to the specific requirements of spiders' leg anatomy. As reduced frequency and low amplitudes of the centre of mass and the angular changes of the body axes are seemingly a result of relatively low leg coordination, strong synchronization of two alternating sets of legs only seems to play a minor role in the locomotion of large spiders.

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11:25 Saturday 30th June 2012

A1.27

Spiders jumping from smooth surfaces: Tarsal attachment during take-off in *Sitticus pubescens*

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Various insects and spiders can jump from smooth surfaces. As classical friction is insufficient and would cause slipping except for very steep take-offs, jumping animals have to adhere to the surface while accelerating. Most animal adhesive structures only make contact when pulled, but jumping usually requires pushing with the hind legs against the typical direction-dependence. Here we investigate how *S. pubescens* spiders perform jumps from smooth surfaces. When leaping onto prey, these spiders accelerate with their third and fourth leg pairs, but the fourth legs only touch the surface during the initial phase of the take-off. We discovered that the different position of both leg pairs results in a different orientation of the tarsus during take-off. While the fourth-leg tips were oriented backward and therefore pushed during take-off, third-leg pretarsi were oriented forward and therefore pulled. This opposite tarsus orientation affected the use of attachment structures. High-speed microscopy of third and fourth-leg tarsi during take-off revealed that the 'pulling' third legs made brief (~9 ms) adhesive contact with their claw tuft setae. By contrast, claw tufts did not come into contact for the 'pushing' fourth legs, but some contact was made by the proximal parts of the tarsus. The adaptations of insects and spiders to jump from smooth surfaces are still poorly understood. Their extremely dynamic attachment organs are promising model systems for studying the mechanisms of adhesion control.

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11:40 Saturday 30th June 2012

A1.28

Joint kinetics of accelerated running over ground *versus* running on an accelerating motorized treadmill belt

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Accelerated running over ground is mechanically different from running on an accelerating motorized treadmill belt. This study documents accelerated running over ground and on an accelerating motorized treadmill belt in 10 young and healthy subjects.

Over ground, ground reaction forces are adapted to accommodate less braking and more propulsion. According to acceleration, the force vector is therefore more forward oriented and the body is more forward inclined during stance. This change in body orientation is partly responsible for the changes in the direction of forces. Next to this, a more pronounced hip and knee flexion at initial contact, a higher hip extension velocity (and peak power generation), smaller knee flexion velocity (and peak power absorption) and smaller initial peak plantar flexion velocity result in less braking. Higher peak knee extension and peak plantar flexion velocity are associated with larger propulsion. Altogether, during stance the joint moments are not significantly influenced by acceleration as such accelerated running over ground might be a consequence of the body in essence doing the same thing but in a different orientation. On a treadmill, ground reaction forces are not influenced by acceleration and only minor kinesiological adaptations to an accelerating belt are observed.

Adaptations to acceleration during running therefore differ from treadmill to over ground and should be studied in the condition of interest.

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11:55 Saturday 30th June 2012

A1.29
Slow but tenacious: A comparative analysis of gripping and running performance in chameleons

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Chameleons are highly specialized arboreal lizards showing a suite of interrelated morphological, functional and behavioural specializations. One of the intriguing morphological features is the specialized hands and feet with opposing digits (three *versus* two) showing varying degrees of fusion. This fusion is considered to be an adaptation to the arboreal lifestyle of chameleons as it allows them to grasp and hold onto narrow substrates, yet several chameleons show a more terrestrial lifestyle and spend considerable amounts of time moving over ground.

Here we test whether terrestrial representatives of two clades of chameleons (genera *Chameleo* and *Bradypodion*) that have independently radiated into more terrestrial habitats differ from closely-related arboreal species in:

- the morphology of the hands and feet;
- external limb and tail morphology; and
- running and gripping performance.

As previous studies have suggested a trade-off between running and gripping in chameleons, we predicted that the more terrestrial species *Chameleo namaquensis* and *Bradypodion occidentale* would show greater running but poorer gripping performance than their arboreal (*Chameleo dilepis* and *Bradypodion damaranum*, respectively) counterparts.

Our data show that species do indeed differ in both morphology and performance, with the more terrestrial species being generally faster yet showing a lower grip force for a given body size than arboreal species. This suggests that chameleons show specialization to habitat use despite their highly conserved blueprint.

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12:10 Saturday 30th June 2012

A1.30
Adaptive locomotion to lateral perturbation in rats, *Rattus norregicus*

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Quadrupedal robots working outdoors are very prone to tumbling down when disturbed by outside forces. Quadruped animals, however, have a significant ability to keep their balance under outside perturbation, even when travelling on rough and irregular terrain. They can even acquire new gait patterns with respect to environmental changes. Here we investigate adaptive locomotion to lateral perturbation in rats, *Rattus norregicus*. Our research aims to formulate a physical model of these rats' behaviour so that we can design a quadrupedal walking robot to imitate such adaptive behaviour.

We studied the ground reaction forces of each leg on substrates with different slopes. We also focused on how rats adjust ground reaction forces and gait under outside perturbation. The ground reaction forces of rats on 0°, 15° and 20° slopes and lateral perturbation on a level surface were measured using force measurement array. The gait behaviour of rats was synchronously recorded by a high-speed camera. The result shows that with increased slope, the hind legs play a greater role in forward movement. While recovering from lateral perturbation, rats can recover their body balance within 300 ms.

The results of this research have exciting possible uses in structure design, gate planning, control development and the anti-perturbation design of quadrupedal robots.

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12:25 Saturday 30th June 2012

A1.31
Dog gait on rough terrain confirms the prediction of a stability inspired dynamical systems model of quadrupedal leg control

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In nature, quadrupeds must maintain stability when moving over uneven terrain in order to survive and reproduce. Gait choice and the maintenance of idealized gaits are likely to be critical for stability, particularly over rough ground. In recent work we have formulated a dynamical systems model of quadrupedal gait that accurately reproduces the relative leg phases over time during the walk, trot and transitions between the two. In this study, we sought independent testing of this model. We therefore tested predictions of the model when experiencing injected noise, by comparing output from simulations of the model with data from freely-running dogs on rough terrain. Model simulations in which random noise was injected at walking speed showed a gait that moved from the idealized walk towards a trot.

To test this prediction, we utilized a wirelessly synchronized sensor suite fitted to five male dogs, of shoulder height 522.0 ±2.6 mm (mean ± standard deviation) and body mass 20.0 ± 2.5 kg (mean ± standard deviation). Devices were attached to the proximal-most segment of each leg, and on the midline of the back at the front legs. Sensor data were used to compute animal speed, position and a continuous estimate of leg phase.

The centroids of relative leg phase (averaged across time within each stride) describing the gait used moved significantly towards trot on rough terrain (linear mixed model; n=5 dogs, $p<0.05$). To explain why we observe these changes, we propose experiments using a physical model, the XRL robot.

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12:40 Saturday 30th June 2012

A1.32
Kinematics of the transition from trot to canter from a dynamical systems perspective in horses

Sandra Nauwelaerts (Antwerp University, Belgium), Peter Aerts (Antwerp University, Belgium) and Hilary M. Clayton (Michigan State University, Michigan, USA)

Animal biomechanics researchers have been fascinated by the existence of different gaits, defined as a sequence of limb oscillations with a specific interlimb coordination pattern that moves the animal. More recently, the focus has shifted towards studying transitions. One approach to the study of transitions is the dynamical systems perspective based on the Haken, Kelso and Bunz (HKB; 1985) model, where changes in coordination patterns are explained by self-organization principles.

The HKB model describes the spontaneous transitions that occur because two preferred states (here gaits) can be seen as stable regions that work as attractors in a stability landscape. A phase transition occurs spontaneously when a control parameter (here velocity) is varied,

prompting a response from the system. Stability is lost when switching between gaits and behaviour is attracted towards a second stable region. A consequence of this model is that it is expected that a transition coincides with a temporary loss of stability that is recovered after the transition.

In this study, we first describe the kinematical changes involved in a trot to canter transition. The forelimb that becomes the leading limb takes a stride that is shorter in duration and length, while the trailing (opposite diagonally) hind limb takes a stride that is longer in length. We also test the HKB model by using joint angle variability as a measure for stability. This approach pinpoints the trailing hind limb as a potential candidate by which to follow the dynamics systems theory and therefore is potentially the joint that determines when the transition will occur.

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12:55 Saturday 30th June 2012

A1.33
Towards a unified notion of gaits

Simon D. Wilshin (Royal Veterinary College, UK), Monica A. Daley (Royal Veterinary College, UK) and Shai Revzen (University of Michigan, Michigan, USA)

While a variety of definitions of ‘gait’ have proved useful in experimental biology, there are many superficially disparate definitions of ‘gait’, both in the literature and among practicing biologists. At times these definitions are contradictory, for example a definition of gait that required a discrete jump in some quantity (as occurs for the walk–trot transition in dogs) could imply that walking and running are indistinct in some birds; yet, these are intuitively different modes of locomotion. Here we propose a unified conception of gait and show how the hierarchical nature of our definition solves this difficulty and highlights important questions relating to control and the determinants of locomotion.

We propose to define ‘gait’ as ‘a parametrically-related family of non-dimensional kinematic observations of a locomotor behaviour that can persist indefinitely’. We arrived at this definition by identifying the commonalities between existing definitions, and highlighting the utility of distinctions, such as the scale-free nature of gait metrics, the cyclic nature of most familiar ‘gaits’ and the natural hierarchies of gaits. We show how existing definitions are embedded in ours. We also highlight the practical utility of our definition in terms of both pedagogy and research.

Our definition conceptually clarifies theories of gait by highlighting the relations between gait hierarchies and locomotion model hierarchies. Our approach sharpens the ability to formulate biomechanical research hypotheses about all forms of locomotion in both species-specific and comparative studies. The resulting language can also be used to describe gaits in non-cyclic locomotion.

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13:50 Saturday 30th June 2012

A1.34
Bigger birds work harder to stand up

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Almost nothing is known about how any animal, except perhaps humans, stands up from a sitting position. Yet this behaviour is not only vital for the daily lives of most land animals but also biomechanically demanding, because it should require near-maximal joint ranges of motion (ROM) and thus greater musculotendinous work, as well as potentially large tissue stresses due to the poor mechanical advantage of a crouched limb pose. There are no general principles formulated for how this behaviour should change with anatomy, size, limb number/posture or other factors that change during ontogeny and phylogeny.

As a first step toward establishing the mechanical principles of the sit-to-stand transition (STST), we hypothesized that larger birds would need to move their limb joints through larger ROM because they would go from similarly crouched to more upright poses. To test our hypothesis, we obtained three-dimensional pelvic limb kinematics from six species of birds, ranging from 0.2 kg quail to 120 kg ostriches using motion-capture or high-speed video (250 Hz), for two to three individuals doing the STST (10 trials per animal with good STST data).

Our findings indicate that the STST strategy varies across bird species, with size-related changes in both individual joint ROM as well as joint coordination (i.e. changes in the relative timing between joints) observed. These changes seem to require relatively greater amounts of mechanical work in larger birds, although joint timing might ameliorate some of these effects.

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14:05 Saturday 30th June 2012

A1.35
Strategies in running: How human runners adapt their centre of mass to visible and camouflaged ground level changes

Michael Ernst (Friedrich Schiller University, Germany), Martin Götze (Friedrich Schiller University, Germany), Roy Müller (Friedrich Schiller University, Germany) and Reinhard Blickhan (Friedrich Schiller University, Germany)

When humans run in nature, they routinely negotiate varying ground and unpredictable perturbations. The strategies they use can be revealed by analysing their centre of mass (CoM). Our first study investigated adaptations while running up and down a single or a permanent visible step of 10 cm height. Our second study focused on adaptations while running across single drops (10 cm in depth) that were either visible or camouflaged, where the drops occurred by chance. For both studies, the relative adaptations in the vertical oscillation of the CoM compared to the undisturbed situation were analysed. We found significant variances in the vertical oscillation for the visible and camouflaged situations. Runners adapt their CoM in preparation for a visible step up or down by lifting it about 50% of step height or lowering it by about 40% of drop height. After the contact on the changed ground level, different adaptations occur depending on the situation (100% for plateau, 60% for a single step up or down). Similar adaptations were found in preparation for a camouflaged step. In the subsequent flight phase, however, the CoM is lowered by about 90% of drop height if runners encounter a camouflaged drop and is almost unaffected if there is no drop. The adaptations show that runners use active strategies to smoothen the CoM trajectory while running on uneven ground. They also indicate that runners rely on passive self-stabilizing mechanisms if the terrain is not accurately predictable, showing that active and passive parts jointly contribute to successful running.

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14:20 Saturday 30th June 2012

A1.36
Three-dimensional biomechanics of the human hip joint: Insight from an integrative analysis of the pelvic-femoral complex

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Humans are characterized by a permanent bipedalism resulting in strong mechanical constraints on the coxofemoral joint. Both stability and mobility are required to ensure a functional interface transmitting forces between trunk and lower limbs. An appropriate three-dimensional orientation of the coxofemoral joint, composed of the acetabulum and the proximal femur, is fundamental to ensure an efficient bipedal gait and posture.

The aim of this study is first to quantify variation in the three-dimensional orientations of both the acetabulum and the femoral neck in adult humans and second to identify patterns of covariation between these two orientations in order to analyse the degree of morphological integration at the hip joint. Results were interpreted using three-dimensional models of the acetabular region and the proximal femur.

Our results show no significant patterns of covariation between the three-dimensional orientation of the femoral neck and the orientation of the acetabulum. In addition, the mean orientations of the two opposing articulating components demonstrated a poor congruity in the normal joint. We suggest that this absence of covariation and this poor congruity might be partly due to the phylogenetic history of the human species. Although natural selection optimizes performance of the human hip joint in the function of locomotion, the adaptation is also dependent on the constraints of the inherited structure and the trade-off with other functions.

To better understand the evolution of the human hip joint and the constraints acting thereupon, we are expanding our study on an extended sample of Hominoids.

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14:35 Saturday 30th June 2012

A1.37
Biomechanics of human bipedal gallop: Asymmetry dictates leg functions

Pieter Fiers (University of Antwerp, Belgium), Dirk De Clercq (Ghent University, Belgium), Veerle Segers (Ghent University, Belgium) and Peter Aerts (University of Antwerp, Belgium)

Unilateral skipping or bipedal galloping is one of the gait types humans are able to perform. In contrast to many animals, where gallop is the preferred gait at higher speeds, human bipedal gallop only shows up in very specific conditions (e.g. fast down-hill locomotion). Whereas walking and running are well-understood gaits, human bipedal gallop is relatively unexplored. This study examines the lower limb mechanics and assesses possible reasons why humans do not spontaneously opt for gallop for steady state locomotion by looking at the kinematics and kinetics.

The kinematic and kinetic data of 12 subjects were collected when galloping and running at preferred speeds. Mechanical work at the lower limb joints was calculated. Analysis revealed that the principal differences between run and gallop are located at the hip. The asymmetrical configuration of gallop involves different hip actions and different foot placing, giving galloping legs different functions: the trailing leg decelerates the body in a vertical direction but propels it forward, while the leading leg does the opposite. Although both legs conserve mechanical energy by interchanging external mechanical energy with potential elastic energy, the anterior–posterior orientation of the legs cause energy dissipation and generation at the hips, making gallop metabolically more expensive compared to run. We therefore conclude that the higher metabolic cost of transport, as well as higher muscular stress at the hips, might be the reason why humans do not use gallop for steady state locomotion.

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14:50 Saturday 30th June 2012

A1.38
Torques in running and heel-sole-toe stance in walking: Striding (slightly) beyond the point-mass view of bipedal gaits

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Reductionist point mass models are useful in understanding the underlying mechanical principles of walking and running. Investigating deviations from the point mass model can be equally insightful. These are, however, incomplete representations of true animal gaits.

Here we consider the implication of two small deviations from pure point mass models. Actual animal muscles cannot produce infinite forces; this means that the gaits predicted to be economical by point mass models are unachievable. There is an unavoidable conflict between mechanically- and physiologically-economical gaits. A further physiological constraint, cost-peculiar to muscles, is a requirement for metabolic work when providing isometric work. The human foot and heel-sole-toe stance allows the calf and shin muscles to be loaded when needed but largely unloaded during the passive vaulting phase. In running, the ground reaction forces pass close to the centre of mass; pitching torques and motions are small. However, sending the ground reduction force through the centre of mass comes at a price: the resulting fore–aft forces require muscle work.

Humans actually run with measurable – albeit small – torques. This allows a near-optimal compromise between reducing kinetic energy fluctuations and avoiding spinning. This is only possible because we are not point masses. With an actual pitch moment of inertia, torques can be resisted to a certain extent without rapidly pitching forwards and backwards. While 'torque-based' energy savings are small in humans, other animals' such as kangaroos with their peculiar long-head/long-tail structure' could benefit considerably from a non-zero centre of mass-torque strategy.

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15:05 Saturday 30th June 2012

A1.39
Innovative hingeless deformation systems inspired by the flower structure of *Strelitzia reginae*

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Biomimetic technical applications are becoming increasingly based on plant movements as concept generators. The present study focuses on a biomimetic actuation and deformation system that is particularly suited for the fields of architecture and constructional engineering. The biological concept generators are the perch in *Strelitzia reginae* (Strelitziaceae). Birds pollinate the flower by landing on a perch and bending it downwards with their body weight. The perch simultaneously opens up, which is a non-autonomous plant movement with external actuation by application of mechanical force. This mechanism is very fail-safe and extremely reliable, as it can be repeated over 3,000 times with no visible deterioration of the perch.

These are also the two main benefits of the biomimetic translation – the Flectofin® – as the avoidance of local hinges (that are also costly due to wear and maintenance) makes it very reliable and fail-safe. The Flectofin is a biomimetic technical structure with a flapping system based on the opening of the perch of *Strelitzia reginae* that was developed using fibre-reinforced polymers such as glass-fibre-reinforced polymers. The good scalability of this biomimetic structure is of high importance as it allows us to develop large individual façade shading elements. The high potential of this structure is proven by its advanced demonstrator status and by being an inspirational platform for the development of another shading system that is currently on display at the World Expo 2012 in Korea.

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15:50 Saturday 30th June 2012

A1.40
Chondrocyte damage in relation to the mechanical micro-environment of the cell

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Mechanical cell damage thresholds are important parameters for numerical models that investigate cell and tissue pathologies and disease. In our current work, we investigate whether cell deformation can serve as a predictor of cell damage, and how the cells' mechanical environment affects cell deformations in a three-dimensional agarose culture system.

Based on earlier work, we hypothesized that cell damage: increases with increasing cell deformation; and increases with increasing deformation duration. Finite element simulations predict that cell deformations will decrease with culture time for equal global agarose strains, because the chondrocytes produce a pericellular matrix over time that is much stiffer than the three-dimensional agarose substrate. We therefore also hypothesize that cells will deform and damage less with increasing culture times.

The three-dimensional culture was subjected to a range of deformations on a confocal microscope to investigate cell damage with real-time viability staining. Histology and biochemical assays were used to quantify cell deformations and the amount of pericellular matrix over culture time. Cell viability decreased with increasing strain and with increasing strain duration. Biochemical assays showed that the amount of matrix increased over culture times; and histology showed that cells deform less with increased culture times for equal global agarose strains.

Thus, the results support our hypothesis that deformation induced chondrocyte damage for equal global agarose strains increases with cellular strains and duration of strains. The results further support the finite element predictions that chondrocytes deform and damage less with increasing culture times.

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16:05 Saturday 30th June 2012

A1.41
Cracking and damage tolerance of the insect wing

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

During the lifetime of a migrating locust, its wings can be subject to deformation, torsion and bending for millions of cycles. How do these insects prevent crack-related material failure in their wings? By observing the growth of cracks in locust hind wings under tension we measured, for the first time, the fracture toughness of this structure. We found that the wing membrane is not particularly tough, however the longitudinal and cross veins act as crack-stopping barriers, inhibiting and preventing cracks from growing through the wing. The presence of veins increases the toughness of the wing by 50%. Using fracture mechanics theory, we showed that the spacing of the wing veins is optimized with respect to the membrane's mechanical properties. This adaptation maximizes the fracture resistance of the wing using a minimum of weight and might inspire the development of biomimetic lightweight and durable materials.

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16:20 Saturday 30th June 2012

A1.42
Structure, function and material relationships in arthropod exoskeletons

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

Arthropods form the most species-rich animal group on earth. One of their evolutionary secrets of success is their exoskeleton, which is made of cuticle. We used engineering design principles to study the relationship between structural shape, functional loading and material properties in two exoskeletal limbs: the tibia of the locust *Schistocerca gregaria* and the merus of the blue crab *Callinectes sapidus*. We estimated the magnitude and direction of forces experienced during strenuous activities (jumping and underwater locomotion, respectively). Using material property data from the literature and our own experiments, we predicted the strength of the limbs under various failure modes.

For the locust we found that jumping creates a state close to pure bending in the tibia. The ratio between the radius and thickness of the tibia was found to be close our predicted optimal value to achieve ideal bending resistance for minimum weight. By contrast, the crab merus during swimming experiences a mixture of bending and axial compression. We found that its radius to thickness ratio represents an ideal compromise in resisting failure under these two different loading modes.

We conclude that evolutionary adaptation has driven these structures' properties towards their optimal values, given their different functional loads and material properties. Interestingly, the same approach applied to the human femur shows it to be far from optimal, having a much lower radius to thickness ratio than the ideal value.

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16:35 Saturday 30th June 2012

A1.43
Attachment forces of a clamp-bearing fish parasite, *Diplozoon paradoxum* (Monogenea)

Wey-Lim Wong (University of Kiel, Germany) and Stanislav N. Gorb (University of Kiel, Germany)

It is well known that monogeneans attach onto their hosts using various haptoral (posterior) attachment devices, but to best of our knowledge no study has been undertaken to estimate the forces generated by the haptoral attachment systems. For the first time, we:

- measured the pull-off forces required to detach a paired adult Diplozoon paradoxum from the fish gills; and
- determined the contribution of muscles to the clamp movements.

An average force of 6.1 ±2.7 mN was required to dislodge a paired *D. paradoxum* vertically from the gills of the common bream, *Abramis brama*. There were significant differences among the widths of clamps openings in the monogeneans treated in three different solutions: the widest clamp openings were observed in the monogeneans treated in 2.5% glutaraldehyde (74.5 ±28.0 µm), followed by the those treated in 20 mM $MgCl_2$ (37.9 ±7.6 µm), and in filtered lake water (20.2 ±8.6 µm). These results suggest that closing of the clamps is due to the elasticity of the clamp material, and not due to the continuous contraction action of the muscles.

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10:25 Sunday 1st July 2012

A1.44
Rapid control of adhesion in smooth adhesive pads of insects

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Many animals can climb on smooth surfaces using directional adhesive footpads. In insects, both smooth and hairy pads allow rapid control of adhesion, as they enlarge the contact area when pulled towards the body but detach when pushed. Directional pads also enable insects to avoid inadvertent detachment by increasing the contact area extremely rapidly when exposed to sudden mechanical perturbations, such as gusts of wind or raindrops. The very short time scale of this reaction (often less than a millisecond) excludes neuromuscular control, so it must be based on passive, mechanical properties of the adhesive system. In smooth adhesive pads of insects, the detailed mechanisms of this reaction are still unclear. The cuticle of smooth pads in insects contains numerous branched fibrils oriented almost perpendicularly to the surface. We hypothesized that, assuming a fixed volume of the water-filled fibrous cuticle, proximal pulls could expand the pad's contact area laterally by reducing the fibril angle. Three-dimensional *in vivo* fluorescence microscopy on the cuticle of smooth stick insect pads confirmed that proximal pulls significantly reduced the fibril angle. The fibril angle variation, however, appeared insufficient to explain the observed increase in contact area. Direct strain measurements in the contact zone demonstrated that pulls not only expand the cuticle laterally (indicating a negative Poisson's ratio of the pad's cuticle), but also added new contact area at the pad's outer edge.

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10:40 Sunday 1st July 2012

A1.45
Holding tight when it gets rough: Adhesion in the northern clingfish *Gobiesox maeandricus*

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The northern clingfish, *Gobiesox maeandricus*, is commonly found in the wave-swept intertidal zones of the Pacific coast of North America. To withstand crushing waves and fast tidal currents, the fish adheres to uneven, slippery and biofouled rocks by using modified pelvic fins as a belly sucker.

Here we measured the adhesive forces with which the fish sticks to surfaces of different roughness, ranging from glass to coarse-grained sandpaper (269 µm). Surprisingly, we found that the fishes adhered equally well to all of the surfaces examined except for the glass surface, on which significantly lower adhesive forces occurred. This result is at odds with our intuition on suction cup performance, which is expected to be best on smooth surfaces and worst on rough substrates.

We directly compared clingfishes to manufactured suction cups. To simulate the effect of fish mucus on suction adhesion, we measured the adhesive forces of manufactured suction cups in fluids with different viscosities. Although we could demonstrate that a highly viscous medium allows manufactured suction cups to adhere to rough substrates, they always failed on the roughest surfaces tested. Adhesion of clingfishes to rough substrates thus cannot be explained by the presence of mucus alone.

By using scanning electron microscopy, we found that papillae in the belly sucker of clingfishes are built from hierarchically-organized hairy structures. We predict that these nanoscopically-sized hairs enhance contact formation along the edge of the suction cup and thus prevent failure of the suction mechanism on rough substrates.

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10:55 Sunday 1st July 2012

A1.46
Sticking like sticky tape: Tree frog acrobatics on overhanging surfaces can be explained by peeling theory

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Tree frogs use adhesive pads on their digits to clamber around in the canopy. Their behavioural strategies for maintaining a grip on vertical and overhanging surfaces are conveniently studied using a rotatable platform.

At angles around the vertical, the frog rotates to face uphill while still maintaining its crouched resting posture with limbs largely tucked in to the body. As the slope becomes more overhanging, the frogs progressively adopt a sprawled posture with the limbs fully extended. The behaviour is dynamic in that limbs tend to slide towards the body due to the animal's mass and have to be re-extended to produce adequate adhesion.

We investigated this change in posture in White's tree frog (*Litoria caerulea*) by measuring the ground reaction forces of individual limbs on a rotatable platform consisting of 24 three-dimensional force transducers. Limb positions were digitized from a synchronized video recording at critical points in the behaviour. When splaying their limbs out sideways, the frogs used opposing feet to generate larger lateral forces pointing towards their body. Reconstructing the angle between the resultant force vector (the vectorial sum of all forces acting on a limb) and the platform showed that frogs in this splayed posture had smaller angles and larger force vectors compared to frogs in a crouched posture. We interpret this behaviour in terms of peeling theory, for both adhesive tape and individual toe pads adhere well at shallow angles of pull, but are easily removed at angles of 90º or more.

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11:10 Sunday 1st July 2012

A1.47
'Quick and easy': Toe pad detachment in the tree frog, *Litoria caerulea*, is fast and effortless

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Tree frogs are capable of adhering to a variety of substrates using their toe pads, and they accomplish this by wet adhesion. This is a temporary adhesive mechanism that is strong yet reversible – properties that are crucial for dynamic activities such as walking and climbing. While a great deal of effort has gone into understanding the attachment mechanism and the functional morphology of the toe pads, less is known about how toe pad detachment occurs. Here we examine the durations, mechanisms and forces involved in toe pad detachment in the tree frog, *Litoria caerulea*, during both walking and climbing on a smooth surface.

Using a recently-developed technique that enables the visualization of the toe pads while in contact with a glass plate, we made high-speed video recordings of changes in pad contact area over time. We found that detachment and, to a lesser extent, attachment are rapid events, particularly during climbing. The recordings also established that the pads

are removed by peeling. This was predominantly from the rear of the pad, but later stages of the process usually involved the sides and occasionally the front as well.

Ground reaction forces exerted by the feet of the frog were measured using a rotatable force plate consisting of 24 individual three-dimensional force sensors. The results demonstrated reproducible patterns of forces that could differ between forelimbs and hind limbs, and between walking and climbing. There was, however, an almost complete absence of detachment forces.

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11:25 Sunday 1st July 2012

A1.48
Fast-starting for a breath: Air breathing in *Hoplosternum littorale*

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Fast starts are brief accelerations commonly observed in fish within the context of predator–prey interactions. In typical C-start escape responses, fish react to a threatening stimulus by bending their body into a C-shape. Recently, similar C-starts have been recorded in archerfish stimulated by the fall of a prey item on the water surface, and in tapping motions of goldfish, a behaviour that was interpreted to be food-related.

Little is known about C-starts being used outside the context of escaping or feeding. Here, we test the hypothesis that air-breathing fish may use C-starts when gulping air at the surface. Air breathing is a common behaviour in many fish species when exposed to hypoxia, although certain species perform air-breathing in normoxia to fill their swim bladders for buoyancy control and/or sound transduction.

Hoplosternum littorale is an air-breathing freshwater catfish found in South America. Field video observations reveal that their air-breathing behaviour consists of a fast air-gulping motion at the surface, followed by swimming towards the bottom. Using high-speed video in the laboratory, we compared the kinematics of spontaneous air-gulping performed by *H. littorale* in normoxia, with those of mechanically-triggered C-start escape responses. Our results show that these two behaviours overlap considerably in their kinematics (turning rates and distance covered), suggesting that air breathing in this species is performed using escape-like C-start motions. This demonstrates that C-starts in fish do not need external stimulation and can be spontaneous behaviours used outside the context of predator–prey interactions.

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11:40 Sunday 1st July 2012

A1.49
Biomechanics meets behaviour: Tail prehension in seahorses

Anabela Maia (Ghent University, Belgium) and Dominique Adriaens (Ghent University, Belgium)

Tail prehension is a common behaviour among seahorses. This behaviour is seen in two related species: the potbellied seahorse, *Hippocampus abdominalis*: and longsnout seahorse, *Hippocampus reidi*, which differ in tail morphology and geographical distribution. *H. abdominalis* has a slender tail and significantly higher number of tail segments (45–48) than *H. reidi* (33–37). *H. reidi* is a tropical and subtropical species while *H. abdominalis* is a temperate species, although both are known to attach to diverse substrates.

We hypothesized that the longer and more slender tail of *H. abdominalis* would perform better than the shorter tail of *H. reidi*. We compared three-dimensional grasping kinematics on a 1 cm horizontal perch between five similar-sized individuals of each species. In *H. reidi*, the whole tail was involved in grasping, although the tail tip had higher displacement, as would be expected. In contrast, in *H. abdominalis* the most proximal third of the tail was not involved in grasping. The linear and angular velocities of the tail tip were similar for the two species. In addition, both species showed lateral bending during tail curling, an unexpected behaviour that might be important for the modulation of grasping in substrates with different orientations, such as corals and seagrasses.

The longer tail of *H. abdominalis* appeared on a first analysis not to be related to increased grasping performance, but it could allow this species to explore substrates with a larger diameter. It could also permit an increase in the feeding radius while attached or play a role in substrate locomotion.

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11:55 Sunday 1st July 2012

A1.50
Hydrodynamic performance of the Boxfish carapace

Klaas Van Manen (University of Groningen, Netherlands), Sam Van Wassenbergh (University of Antwerp, Belgium) and Eize J. Stamhuis (University of Groningen, Netherlands)

Previous work by Bartol and co-workers indicated that the shape of the bony carapace of boxfish (Ostraciidae) plays a significant role in hydrodynamic stability during swimming. Their flow tank measurements and flow visualizations showed specific vortical flow patterns that were argued to be responsible for course self-stabilization: when the animal pitches or yaws a certain angle with respect to the swimming direction, fluid forces exerted on the carapace would cause stabilizing moments about its centre of mass, reorienting the fish parallel to the flow. Additionally, drag coefficients reported for certain species (e.g. 0.1 for *Lactophrys triqueter*) are remarkably low for such poorly streamlined shapes.

Both swimming-direction self-stabilization and a low body drag fit best to migratory swimmers, whereas boxfish usually are slow speed manoeuvrers of tropical reefs. To test this apparent contradiction, flow tank drag force and turning moment measurements of realistic body models and computational fluid dynamics simulations were performed for the species *L. triqueter* and *Ostracion cubicus* at a series of yawing and pitching angles of attack (Re = 6.5 × 104).

Both the empirical and computational approach found strong destabilizing moments instead of stabilizing moments, thus rejecting the self-stabilization hypothesis. The minimal drag coefficient for *L. triqueter* was considerably higher than reported earlier (Cd = 0.27), the minimal Cd for *O. cubicus* being of similar magnitude (Cd = 0.29). These results indicate that the shape of the boxfish carapace promotes manoeuvrability, and any stabilization must be controlled by action of the fins.

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12:10 Sunday 1st July 2012

A1.51
Triangular and sinusoidal patterns of strain during dogfish swimming

Roger C. Woledge (Royal Veterinary College, UK) and Nancy Curtin (Imperial College London, UK)

Scyliorhinus canicula (small-spotted catshark or dogfish) were videoed from above while spontaneously swimming in a large circular tank at the Aberdeen Marine Laboratory. The water temperature was 12ºC. We selected portions of the video showing fish swimming steadily at typical cruising rates: approximately 0.7 body lengths per second with a tail beat frequency of about 0.7 Hz. In successive frames of the 30 Hz video, we measured the profile of the fish body, omitting the fins, and calculated the position of the midline.

From the curvature of the midline and the width of the fish, we calculated the strain (proportional change of length) of the lateral surfaces. The maximum strain within each cycle of movement was between 5 and 10%. This slow swimming is probably powered by the red muscle fibres that run parallel to the long axis of the fish and are located close to the lateral surface. These muscle fibres thus experience an overall length change of 10–20% during slow swimming. Near to the head and tail, the pattern of length change is approximately sinusoidal, but for most of the fish body the time course of the muscle fibre length change is a symmetrical triangular wave.

We thank Dr Clem Wardle of the Aberdeen Marine Laboratory for providing us with these videos of swimming dogfish.

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12:25 Sunday 1st July 2012

A1.52

Novel mechanisms in larval zebrafish C-starts: An integrated study of body dynamics and hydrodynamics

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Fishes swimming in body and caudal fin (BCF) mode often exhibit stable body undulation during cyclic swimming, but can also perform remarkable manoeuvrability with significantly different swimming styles in the case of C-start. The kinematics of C-starts has been studied extensively, yet few hydrodynamic studies have been conducted so far on C-starts.

Aiming to unveil the mechanisms of swimming hydrodynamics and manoeuvrability of C-start, we developed an integrated three-dimensional computational approach to hydrodynamics and free-swimming body dynamics that couples the Navier-Stokes equations to the equations of undulating body motion and focused on larval zebrafish (*Danio rerio*), which swims in an intermediate Reynolds number regime ($100 < Re < 1{,}000$).

A key validation is discussed by comparing computational predictions with two-dimensional particle image velocimetry experimental measurement on an entire burst-and-coast swimming bout with C-start. This revealed that the model accurately predicted the fishes' centre-of-mass motions as well as the spatial and temporal characteristics of the generated flow patterns.

Furthermore, our simulations cover extremely fast C-start manoeuvres in conditions of emergency escape. Within tens of milliseconds, the preparatory stroke of C-start can results in a change of more than 180º in heading angle, then the propulsive stroke provides sharp acceleration for flee.

Our computational approach can tolerate dramatic body bending during C-start, even if the fish shapes itself into a circle or even with its head and tail are crossed. Based on the similarity between computational and experimental results, we are seeking further information and phenomena so that we can devise a better explanation for the mechanism behind this lightening manoeuvre.

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12:40 Sunday 1st July 2012

A1.53

Dynamics of high-speed locomotion and hunting in free ranging cheetah

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Studies of maximum performance are limited by subject motivation and attempts by us and others to measure domestic cheetah performance show limited straight-line and manoeuvring performance. We set out to describe the speed, acceleration and manoeuvring of wild cheetahs when hunting. We developed a collar powered by a combination of rechargeable batteries, non-rechargeable batteries and solar panels. Sensors comprise a 5 Hz L1 pseudorange Doppler data GPS receiver, three-axis microelectromechanical systems (MEMS) accelerometer, three-axis MEMS gyroscope, and a three-axis magnetometer. Data were off-loaded via a wireless link to an aircraft or vehicle. At 300 Hz the sensors provide acceleration (force), integration velocity and position, angular velocity, heading and orientation of the collar and (approximately) the position of the cheetah.

GPS and inertial measurement unit data are fused using our own Kalman filtering optimized for sensor characteristics and animal dynamics to provide the data we require. The collar adapts its operation (and hence power consumption) across six states depending on the time of day, the animal's activity level and battery voltage. This allows the collection of fine-grained behaviour and movement data and therefore produces unbiased records of hunting behaviour. Collars were attached to three cheetahs in the Okavango Delta area of Botswana. To date we have collected data for 129 runs from these three cheetahs and data collection is ongoing. Successful hunts involve rapid acceleration and deceleration, indicating high muscle powers, relatively high-speed galloping and a period of manoeuvring with high lateral accelerations.

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12:55 Sunday 1st July 2012

A1.54

Modulation of food capture in *Iguana iguana* (Iguanidae, Squamata)

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Although many phenotypic traits present some plasticity, behaviour is often considered one of the major 'plastic' aspects of phenotype. It is widely accepted that modifications in behaviour are the quickest response to temporal changes in the environment. Constraints on sensory ability, cognitive properties and physiological capacities limit this behavioural plasticity but balance between plasticity and relative consistency in behaviour is feasible.

The kinematics of food catching in *Iguana iguana* was compared with various food items (fruits with highly different textures including apple, tomato and banana, and mice) to determine the phenotypic functional and behavioural plasticity in lingual prehension. All food offered was of a similar size and volume, but different in surface texture. Variables depicting head, jaw and tongue movements during food prehension were digitized from high-speed video records obtained from adult specimens.

The dataset reveals a difference in behavioural traits in regards to the textural properties of the food. Lingual and jaw prehension drastically

differed for each type of food, suggesting a complex adaptive response to food texture in this squamate. Humidity on the surface of the food item plays a key role in the selection of prehension mode by *I. iguana*. Lingual prehension occurs for food with a dry surface.

Variation in the phenotypic behavioural traits in *I. iguana* faced with various food resources is suggested to be an adaptive plasticity, with local habitat differences driving the selection of behavioural and functional traits relevant in a given ecological context.

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Poster Session – Saturday 30th June 2012

A1.55
Selective recruitment of muscle fibre types *in situ*

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The diversity of locomotor functions seen in many animals requires muscle to produce a wide range of mechanical outputs. The appropriate recruitment of different muscle fibre types for specific locomotor functions greatly contributes to this. Muscle fibre types are defined by their distinct biochemical and contractile properties, which result in different force–velocity properties and force rise and relaxation times. The recruitment of different fibre types therefore has profound implications for the mechanical output of a muscle.

The mechanical output of muscle in response to electrical stimulation has been measured in isolated muscle preparations for over a century. This has taught us much about muscle function, such as the relationship between muscle length and shortening velocity and the force it can produce, and the advent of the work-loop technique has allowed us to investigate the mechanical implications of *in vivo* muscle function. These techniques rely on the supramaximal activation of the muscle preparation, however, and thus prevent investigation of the mechanical implications of the recruitment of different muscle fibre types.

In this study, rat plantaris muscles were stimulated *in situ* via the sciatic nerve and the mechanical output was recorded. A blocking electrode was used to selectively block faster motor units, thus allowing the mechanical output of the muscle to be recorded with varying degrees of recruitment. This technique will allow more accurate replication of *in vivo* muscle function *in vitro*, and will contribute to improved muscle models by allowing information on fibre type to be incorporated into Hill-type muscle models.

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Poster Session – Saturday 30th June 2012

A1.56
The energy cost of jumping

Lewis G. Halsey (University of Roehampton, UK), Samuel R.L. Coward (University of Birmingham, UK) and Susannah S.K. Thorpe (University of Birmingham, UK)

The energetic cost of horizontal jumping in humans was compared on surfaces with different properties. Three surface types were used – 'firm' (F), 'compliant' (C) and 'dampened' (D) – to generate five experimental conditions: F to C; C to F; F to F; C to C; and D to D. All five conditions were tested at a jumping distance of 180 cm, while three of the conditions were also tested at 120 cm.

For each condition, participants jumped at 0.2 Hz, back and forth between surfaces, for about three minutes while wearing a portable respiratory gas analyser. For jumps of 120 cm there were no differences in the cost (mL O_2 m^{-1}) between the three conditions tested. For jumps of 180 cm, there were several significant differences between the five conditions. Both F to C and F to F conditions were more costly than C to F and C to C. D to D was more expensive than all other conditions. The findings of this study suggest that:

- jumping from a firm surface can be more energetically costly than jumping from a compliant surface;
- landing on a firm surface *versus* a compliant surface does not affect the cost of jumping;
- the energy saving experienced by jumping from a compliant *versus* a firm surface is only present when jumping large distances;
- jumping to and from a dampened surface is more costly than jumping to and from compliant or firm surfaces.

The likely reasons for these findings are discussed.

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Poster Session – Saturday 30th June 2012

A1.57
Effects of dragline silk on jumping performance of the jumping spider (*Hasarius adansoni*)

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Spider silk has long been known to be a remarkable material due to its outstanding mechanical properties (e.g. great extensibility, strength and toughness) and for various functions, including prey capture, dispersal, as a safety line or even a brake. To date, most studies on spider silks have been based on web-building spiders, and only a few have considered the roles of silks on wandering spiders that cannot construct a web. Here we examined the effects of dragline silks on the jumping performance of jumping spiders, *Hasarius adansoni*, by comparing normal subjects with those did not produce silk.

We filmed each spider during a jump using high-speed video camera at 1,000 frames per second, and analysed jumping kinematics throughout the whole process. The results suggest that during a jump the dragline silk can help decrease a spider's landing velocities. We estimated that about 45% of the original mechanical energy (sum of potential and kinetic energy) was absorbed by the silk extension, and about 30% was dissipated by drag. We also observed that without dragline silk, the spider landed in a more upright posture and would slip or tumble whereas spiders with silk could adjust body orientation using silk tension and land in a better posture for foot–ground contact.

In conclusion, the dragline silk of a jumping spider not only slows the animal but also stabilizes the body during the aerial phase of jumping.

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Poster Session – Saturday 30th June 2012

A1.58
The effects of wing morphology and body mass on take-off performance in blue tits (*Cyanistes caeruleus*)

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Take-off is a crucial component of the flight behaviour of birds as it is the means by which they get airborne and is important in avoiding predation. We investigated the effects of wing morphology on escape take-off

performance in blue tits (*Cyanistes caeruleus*).

High-speed video recordings were used to track the position of the centre of mass and the sum of the rates of change of the kinetic and potential energies was calculated to determine take-off performance. Intra-specifically, wing morphology varies as a result of moult, feather condition and age. Body weight changes both diurnally and seasonally, primarily as a result of body fat deposition. Wing morphology is a major determinant of lift and thrust generation and should have an impact on take-off performance, however this has not been convincingly demonstrated. The effects of wing morphology in relation to body weight during take-off performance were determined using principle component analysis and linear regression.

Individuals with a low wing loading (ratio of body weight to wing area) took off at higher velocities and had increased take-off performance. The blue tits in this study with high wing loading and reduced take-off performance also had a low wing area; this was related to feather moult. High wing loading was also due to high body weight; potentially due to increased body fat deposition.

Maintenance of feather condition through moult and reducing the risk of starvation by depositing body fat could therefore potentially increase the risk of predation due to impaired take-off performance.

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Poster Session – Saturday 30th June 2012

A1.59

Power output of skinned skeletal muscle fibres from the cheetah (*Acinonyx jubatus*)

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Muscle bundles were dissected from the gluteus, semitendinosus and longissimus muscles of a captive cheetah euthanized at the Royal Veterinary College, UK. Single muscle fibres (1.5 mm long) were dissected from permeabilized muscle bundles and mounted between T-shaped clips. Fibres were activated using a temperature-jump protocol; 0°C followed by a rapid jump to 20°C at which force develops. The force and length changes before, during and after shortening were recorded at 20°C. For direct comparison, the same protocol was used with fast psoas fibres from rabbit. Each fibre was activated up to six times. After isometric force had developed a step and ramp shortening was imposed at a velocity of up to seven fibre lengths s^{-1}. Power was evaluated as the product of force and shortening velocity of the contractile component. The force and power *versus* velocity relationships were very similar for cheetah (n=15, 3 outliers were rejected) and rabbit psoas (n=7). The maximum power values were similar to those reported for intact, fast fibres from rat at 20°C (He *et al*., *J Physiol.*, 1999; 517: 839–854). At 20°C, the maximum power that we measured was 4.3 times greater than that produced at 12°C by skinned fibres from rabbit (He *et al*., *J Physiol*., 1999; 517: 839–854) and rat (Bottinelli *et al*., *J. Physiol.*, 1991; 437: 655–672). This reflects the strong temperature dependence of power output in the temperature range 10 to 20°C reported by Ranatanga (*Exp. Physiol.* 1998; 83: 371–376).

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Poster Session – Saturday 30th June 2012

A1.60

Does the ergogenic benefit of caffeine change with age? The effect of physiological concentrations of caffeine on the power output of maximally stimulated mouse EDL and diaphragm muscle

Jason Tallis (Coventry University, UK), Rob S. James (Coventry University, UK), Val M. Cox (Coventry University, UK) and Michael J. Duncan (Coventry University, UK)

The effects of caffeine as an ergogenic aid have been widely studied in mature human populations with well-established improvements during endurance and high-intensity exercise regimes. The potential age-related ergogenic response of caffeine has, however, received far less attention and could be an important area of investigation due to age-related changes in muscle composition.

The present study aims to build upon previous work using the work loop technique to assess the direct effect of 70 µM caffeine (physiological maximum) on the maximal power output of isolated mouse EDL and diaphragm muscle from four different age groups (three, 10, 30 and 50 weeks). In both mouse EDL and diaphragm muscle, 70 µM caffeine treatment resulted in a significant increase in maximal muscle power output that was generally greatest at 10 and 30 weeks (up to 5% and 6% improvement, respectively). This potentiation of maximal muscle power output was significantly lower at 50 weeks (up to 3% and 2% improvement, respectively) and in mice in the development stage at three weeks (up to 1% and 2% improvement, respectively).

The previously-established muscle-specific caffeine response appears to be prevalent in skeletal muscle but is likely affected by both muscle development and age-related sarcopenia. Consequently, caffeine could be used to enhance muscle force in the elderly; however this established effect might be significantly reduced when compared to skeletal muscle from younger individuals.

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Poster Session – Saturday 30th June 2012

A1.61

A three-dimensional geometric morphometric study of the avian sternum

Débora Martínez-Salmerón (University of Barcelona, Catalonia, Spain), Eloy Gálvez-López (University of Barcelona, Catalonia, Spain) and Adrià Casinos (University of Barcelona, Catalonia, Spain)

The shape variation of the avian sternum of 26 species of flying birds was analysed using three-dimensional geometric morphometrics. The species belonged to different orders and differed in their ecomorphology and flying type. A total of 24 landmarks were digitized from each specimen. After a general procrustes alignment, a principal component analysis and several canonical variates analyses were carried out with the partial warps in order to describe sternal shape variation and to analyse the clustering of the specimens in terms of functional adaptations to habitat and flight type.

Size had a significant effect on avian sternum shape variation (40.68%, p<0.0001), but most of it was caused by size differences between families, since the allometric effect decreased to 15.41% (p=0.0024) after removing the phylogenetic effect using phylogenetically-independent contrasts. We found a shape gradient from semi-aquatic birds, to land gliders (Corvidae, Falconidae, Strigidae), to forest-dwelling small passerines with fast flapping flight. Semi-aquatic birds presented a narrow sternum with short, caudally-displaced processes and a cranially projected keel. Furthermore, the cranial apex of their keel exceeded the sternal body, which has already been related to long-distance flight and diving capabilities by Drake *et al*. (2009). Birds with fast flapping flight had

a wider sternum with long, cranially-displaced processes, which probably enhances flying manoeuvrability, and a caudally displaced keel whose apex did not exceed the sternal body, typical of short-distance flyers (Drake *et al*. 2009).

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Poster Session – Saturday 30th June 2012

A1.62
Insights into the structure and function of tree frog adhesive toe pads obtained from cryo-scanning electron and atomic force microscopy

W. Jon. P. Barnes (University of Glasgow, Scotland, UK), Martina Benz (University of Kiel, Germany), Henrik Peisker (University of Kiel, Germany) and Stanislav N. Gorb (University of Kiel, Germany)

This comparative cryo-scanning electron and atomic force microscope study of the Malaysian flying frog, *Rhacophorus prominanus* (Family Rhacophoridae), and White's tree frog, *Litoria caerulea* (Family Hylidae), has enabled us to compare pad surface micro- and nanostructures, cytoskeletal structures and frozen footprints in two distantly related species, where toe pads can be presumed to have evolved independently.

Overall, we have found extraordinary similarity between structures in the two species examined. Given their independent origins, this suggests that there is an optimal structure for a toe pad, which might have implications for biomimetics. The epithelium is multi-layered, the outer layer being shed at intervals. Such frequent replacement of the outer surface avoids problems of wear. The cells are mostly hexagonal, with deep channels between them. This allows the cells to form their own close contact with surface irregularities. In our footprint studies, these channels were seen to be fluid-filled. The fluid extended all over the ventral surface of the pad, ending in a thicker layer around the pad perimeter. This is surprising, as it would reduce the capillarity forces that are important for toe pad adhesion. An array of nanopillars covers the surface of these cells, each nanopillar having a concave top. They originate from desmosomes and are thought to play an important role in friction. The most prominent of the cytoskeletal structures are keratin fibrils that lie at an angle to the surface. They extend inwards from the nanopillars and presumably promote good adhesion by affecting toe pad compliance.

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Poster Session – Saturday 30th June 2012

A1.63
Friction forces of insect claws on rough surfaces and implications for the grip of pollinators on flower petals

Alexander Hackmann (University of Cambridge, UK), Werner Baumgartner (RWTH Aachen, Germany), Beverley Glover (University of Cambridge, UK) and Walter Federle (University of Cambridge, UK)

Tarsal claws are essential for allowing insects to grip on rough surfaces, but little is known about their detailed performance. We performed friction force measurements with single claws of bumblebees (*Bombus terrestris*) on surfaces of different roughness, and tested the effects of normal force, claw angle and wear.

Claw friction forces increased with normal force and for larger-scale surface roughness. Forces depended on the relationship between claw sharpness (measured by the claw tip radius using scanning electron microscopy) and asperity size. For smaller angles between claw tip and substrate, claws exhibited stronger direction-dependence, with forces during pulling strongly exceeding those during pushing. After multiple sliding cycles on a rough epoxy substrate, wear was visible on the claw tip and claw forces were decreased as a result of the larger claw tip radius.

We tested the idea that pollinating bumblebees use their claws to grip on flower petal surfaces, many of which possess a characteristic microstructure of conical epidermal cells. Friction measurements of bumblebee claws on epoxy replicas of selected flower petal surfaces showed that forces were significantly higher for petals with conical cells than for those without. Thus, conical cells of flower petals provide a suitable topography to allow the claws of pollinating insects to grip.

Further work is needed to explore the detailed mechanics of claw–surface interactions and to understand the effects of morphological variation between claws of different insects and their implications for insect–plant interactions.

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Poster Session – Saturday 30th June 2012

A1.64
Studying the effect of surface roughness on insect adhesion using microstructured substrates

Yanmin Zhou (University of Cambridge, UK), Adam P. Robinson (University of Cambridge, UK) and Walter Federle (University of Cambridge, UK)

Many insects are capable of climbing on natural surfaces of various roughnesses; however, the details of how surface roughness affects insect adhesion are currently unclear. Natural rough substrates are opaque and usually have complex topographies with asperities at multiple length scales, making both observation and experimental manipulation difficult. To overcome these difficulties, we fabricated transparent substrates with well-defined microstructures using photolithography and nanoimprinting.

Two sets of epoxy substrates were manufactured, with square arrays of pillars of 0.5 or 1.4 µm height and spacings ranging from 3 to 22 µm. On these substrates, we visualized the adhesive contact area of smooth pads of cockroaches (*Nauphoeta cinerea*) and hairy pads of dock beetles (*Gastrophysa viridula*) using interference reflection microscopy.

Both cockroach and beetle pads made full contact on the substrates with 0.5 µm pillars, but achieved only partial contact to the tops of the pillars on the densest arrays with 1.4 µm pillars. Single-pad force measurements showed that the transition from partial to full contact is accompanied by a significant decrease in shear force. While the forces for smooth cockroach pads were fully explained by this transition from partial to full contact, the forces of hairy pads decreased, even for the dense arrays of the shorter pillars where they appeared to make full contact.

Our work shows that microstructured substrates are a powerful tool by which to clarify the mechanisms of insect adhesion on rough surfaces and to reveal performance differences between smooth and hairy pads.

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Poster Session – Saturday 30th June 2012

A1.65
Development of an inertial and aerodynamic measurement system for use on birds of prey

Kate V. Reynolds (Oxford University, UK), Graham Taylor (Oxford University, UK) and Adrian Thomas (Oxford University, UK)

Birds are able to exploit atmospheric energy and negotiate the gustiest of conditions with great skill, efficiency and apparent ease. There is an abundance of potential lift sources in the environment, ranging from thermals and ridge lift to wind shear and small-scale turbulence. Utilizing these energy sources is challenging due to the unpredictable and turbulent nature of the atmosphere. Through detailed measurements of the flight dynamics and performance of trained birds of prey using on-board inertial instrumentation, we aim to improve our understanding of

how they deal with and exploit atmospheric heterogeneity. Similar soaring strategies can be applied to manned and unmanned air vehicles with the aim of increasing their flight endurance, reducing fuel consumption and ultimately enabling autonomous flight in unexplored terrain.

Details of instrumentation developed for onboard flight analysis are presented here. Such a system is technically challenging, as the sensors must not only be robust to operate in harsh conditions on the back of a bird, but size and weight must also be minimized to prevent obstruction to the birds' flight. Essential data include accurate measurements of attitude, airspeed, altitude and flight path. These are obtained using integrated GPS, pressure transducers, barometers and a fusion of gyro, accelerometer and magnetometer sensors. The instrumentation was tested on a Steppe eagle soaring in thermal and ridge lift in Wales.

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Poster Session – Saturday 30th June 2012

A1.66
Effect of substrate wettability on attachment performance in smooth adhesive pads of insects

David Labonte (University of Cambridge, UK) and Walter Federle (University of Cambridge, UK)

Insect footpads attach to surfaces via small amounts of fluid secreted into the contact zone. In some insects, this adhesive secretion consists of small aqueous droplets dispersed in an oily continuous phase. We studied for smooth attachment pads of stick insects (*Carausius morosus*) to determine how substrate surface energy influences both the structure of the adhesive emulsion in the contact zone and the friction forces generated by the pads.

Five smooth surfaces covering the range from hydrophilic to hydrophobic (water contact angles 0° to 110º) were produced by depositing monolayers of octadecyltrichlorosilane on glass cover-slips and subsequently exposing them to oxygen plasma for different times. Interference reflection microscopy of stick insect adhesive pads in contact with these substrates revealed that the abundance of large, watery droplets increased for more hydrophilic substrates, whereas hardly any aqueous droplets were visible on the most hydrophobic surface. This effect might be based on a higher tendency for the droplets to coagulate on hydrophilic substrates.

By measuring the shear stress of single adhesive pads on these substrates, we found that attachment pads generated smaller forces on both strongly hydrophobic and hydrophilic surfaces, but forces were maximal on substrates with intermediate wettability. This suggests that the shape and size distribution of the aqueous droplets influences the shear resistance of the secretion. The surface energy range in which smooth pads generate high forces might be adaptive, as it roughly corresponds to the wettability of natural plant surfaces.

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Poster Session – Saturday 30th June 2012

A1.67
Scaling of power output during escape swimming in scallops

Graham N. Askew (University of Leeds, UK) and Marion Kauffmann (University of Leeds, UK)

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 (Mb) according to either geometric or elastic similarity (as Mb-0.33 or Mb-0.125, respectively). Few empirical studies have been conducted that allow these hypotheses to be tested.

We measured muscle mass-specific power output *in vivo* during escape swimming in two species of scallop that covered a range of body masses. Muscle mass-specific power decreased with increasing body mass, scaling as Mb-0.119. Muscle stress, strain and consequently muscle mass-specific work were approximately constant across the range of body masses studied, and therefore mass-specific power was largely determined by the scaling of muscle contraction frequency with body mass, which scaled as Mb-0.155.

These results are consistent with scaling of muscle mass-specific power according to elastic similarity rather than geometric similarity.

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Poster Session – Saturday 30th June 2012

A1.68
Maximizing the length of a single leap step in human walking and running at a range of speeds

Katherine Daniels (University of Bristol, UK) and J.F. Burn (University of Bristol, UK)

A leap step can be implemented during terrestrial locomotion to traverse a region of ground that is unviable for foot placement. Theoretical models and experimental results have shown a positive relationship between approach speed and long jump distance. The aim of this study was to investigate the dependency of leap step length on gait speed.

Human subjects travelled along a straight, level 20-m track at one of five speeds, ranging from walk to maximum-speed run. A maximum-length leap step was incorporated into each trial. Kinematic data were recorded using optical motion capture and combined with published anthropometric data for analysis.

Longer leaps were achieved with faster approaches, but the increase in step length from the ultimate approach step to the leap step was invariant with approach speed. At all submaximal approach speeds there was a net gain in mechanical energy during take-off stance phase and the leap step speed was greater than the approach speed. At the fastest approach speeds, there was no significant net change in mechanical energy but a lengthened step was achieved by redirecting momentum.

The mechanical energy changes suggest that there are two mechanisms involved in maximizing the length of a leap step: at low approach speeds, energy is added by muscular work during the take-off stance phase; whereas at high speeds the redirection of momentum might be explained using the dynamics of a planar spring mass system and is similar to that observed during human long jumps (Seyfarth *et al*., *J. Biomech* 1999; 32: 1259–1267.).

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Poster Session – Saturday 30th June 2012

A1.69
The effect of three-dimensional wing shape on gliding aerodynamics in dragonflies

Toshiyuki Nakata (University of Oxford, UK), Per Henningsson (University of Oxford, UK), Hao Liu (Chiba University, Japan) and Richard J. Bomphrey (University of Oxford, UK)

The corrugations in dragonfly wings stiffen them against high aerodynamic and inertial bending moments. Corrugations can also directly or indirectly enhance the aerodynamic characteristics of the wings by modifying lift to drag ratios or enabling high aspect ratios – thereby reducing induced drag – without greatly increasing material volume or torsional stiffness.

The aerodynamic effect of insect wing corrugations in gliding or flapping flight has been reported previously with most analyses performed in two-dimensions, overlooking the consequences of span-wise variation

in corrugation pattern and three-dimensional aerodynamic effects. Three-dimensional analyses have been limited to the extrusion of a common corrugated profile along the wings' length.

In this study, a computational fluid dynamic analysis of gliding dragonflies was performed using a selection of three-dimensional wing shapes measured as a series of cross-sections along the wing span, given by projecting a laser light sheet onto the wing surfaces of museum and recently-captured dragonfly specimens. Corrugation varies greatly along the longitudinal axis and its effect on aerodynamic performance was evaluated by comparing the full-fidelity models with alternative, artificial, wing shapes such as smoothed or flat wings. Our results describe the importance of corrugations in dragonfly wings in terms of gliding aerodynamics.

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Poster Session – Saturday 30th June 2012

A1.70
Running on different surfaces – kinematics and neural control of two cockroach species (*Periplaneta americana* and *Blaptica dubia*)

Florian Hoffmann (Biomimetics Innovation Centre, University of Applied Sciences, Germany), Chris Dallmann (University of Bielefeld, Germany) and Antonia B. Kesel (Biomimetics Innovation Centre, University of Applied Sciences, Germany)

We investigate the kinematics of two cockroach species that are running on different substrate types. Cockroaches have a stable and adaptive locomotion in complex and changing terrain with a rather simple neuronal control system. We focus on how different neuronal control strategies (centralized *versus* decentralized) adapt to substrate changes.

In order to analyse the time cockroaches need to adapt their gait to a new substrate, we tested different substrate configurations (hard–loose ground; hard ground–2 cm loose ground–hard ground; hard ground–4 cm loose ground–hard ground). Homogeneous loose ground was created using micro glass spheres with a diameter of 200–300 µm. The runs were high-speed videotaped (1,000 frames per second) and the dorsal and sagittal positions of head, abdomen and dorsal tarsal positions were tracked. We used a kinematic phase analysis that allows us to calculate frequency and phase for every instant of the stride cycle and provides a high temporal resolution investigation of gait changes. Onsets and durations of phase or frequency changes can then be used to analyse the underlying neural control architectures.

We hypothesize that within the velocity range of the tripod gait, cockroaches mainly recover passively from small loose substrate perturbations (short contact times), whereas the level of neural feedback increases with larger perturbations (longer contact times), independent of the phase at which the perturbation is introduced to the system.

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Poster Session – Saturday 30th June 2012

A1.71
Foot grip force in young and elderly individuals

Junichiro Yamauchi (Tokyo Metropolitan University, Japan)

A deterioration of skeletal muscle function is the main consequence of ageing. The impairment of neuromuscular function in lower limbs might affect functional movement of daily living and cause a fall after stumbling in elderly individuals. The foot bears the body weight as it carries the body through daily activities, so it plays an integrative role in controlling posture and movements. There are numerous studies on muscle functions of lower limbs; but few studies have demonstrated how foot grip force changes with ageing. The present study investigated the generation of isometric foot grip force in young and elderly individuals.

The maximum foot grip forces of 19 healthy young (21.1 ±1.8 years old) and 18 healthy elderly (66.3 ±6.9 years old) individuals were measured on the dynamometer. For the measurement of maximum isometric foot grip force, the individuals exerted force with a maximum effort for ~3 s on a foot grip dynamometer in a sitting or standing position. Measurements were repeated three times with at least a one-minute rest period between bouts, and the highest value among the measurements was used.

Foot grip forces in the sitting position were significantly lower in older compared to young individuals; but in the standing position these forces were not significantly different.

The results indicate that some form of compensation enables older individuals to maintain a high foot grip force in a standing position.

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Poster Session – Saturday 30th June 2012

A2 – BIOMECHANICS OF LOCOMOTION IN FLOCKS, SCHOOLS AND SWARMS

A2.1
Vorticity and flow patterns for energy conservation of organized formations

Frank E. Fish (West Chester University, Pennsylvania, USA)

As metabolic energy is a limited commodity, strategies that are used by animals to reduce energy costs have important benefits. Locomotion incurs high energetic costs, particularly when swimming through the dense and viscous aquatic medium or having to work against gravity during aerial flight. Various animals organize into defined aggregates while traveling (e.g. schools, pods, flocks, queues, herds and swarms). Movements in organized formations during swimming and flying have been hypothesized to reduce an individual animal's energy expenditure.

Although formations involved with swimming and flying appear to be functionally different, the mechanisms for energy conservation are related by the mechanics of the fluids associated with the flow dynamics. The energy shed into the wake by a leading animal as vorticity affects the pressure and velocity fields experienced by trailing individuals. The pattern of vorticity determines the optimal configuration of the formation. These formations include the V-formation of flying birds, diamond arrangement of fish schools, echelon formation of cetaceans and single-file arrangement of swimming ducks. Vortices induce relative velocities that are advantageous for trailing individuals to reduce drag or enhance lift. In cases of close proximity between adjacent individuals, flow can be channelled to increase flow velocity and generate attraction forces for drafting.

The proportion of an animal's overall energy budget associated with locomotion is high and thus moving in a coordinated manner within discrete formations can lower locomotor costs by taking advantage of induced flow conditions.

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09:10 Monday 2nd July 2012

A2.2
Fish positions relative to neighbours modulate the hydrodynamic advantages of schooling

Stefano Marras (Istituto di Scienza e Tecnologie dell'Informazione 'A. Faedo', CNR, Italy), Shaun S. Killen (University of Glasgow, Scotland, UK), David J. McKenzie (CNRS, France), John F. Steffensen (University of Copenhagen, Denmark) and Paolo Domenici (CNR, Italy)

Schooling behaviour is a widespread phenomenon shared by a large number of fish species. One of the most common benefits of swimming in a school is the hydrodynamic and energetic advantage obtained by its members. Fish occupying non-frontal positions can benefit from the flow generated by the caudal movement of fish swimming in the front.

While previous work has demonstrated that trailing fish show a lower tail beat frequency (TBF) than leading fish, the extent to which schooling provides hydrodynamic advantages compared to swimming alone has not been quantified. We quantified this by filming individual grey mullet *Liza aurata* when swimming alone (n=20) and the same fish when swimming in a school (n=20; eight fish per school, with one focal fish in each school) at three swimming speeds in a swim-tunnel. TBF was measured in focal fish swimming to the side of a neighbour, using a range of distances along the direction of locomotion, spanning one body length (BL) in the front (+1BL) and behind (-1BL) a neighbouring fish.

We found a significant reduction in the mean TBF of fish when swimming in a school *versus* solitary fish. Furthermore, the TBF of the focal fish decreased linearly between the two extreme positions (from +1BL to -1BL), with the lowest values at -1BL.

This work provides direct evidence that schooling provides hydrodynamic advantages compared to solitary swimming and that small changes in the position of an individual fish relative to neighbours modulates these advantages.

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10:30 Monday 2nd July 2012

A2.3
Energetics of swimming of schooling fish

John F. Steffensen (University of Copenhagen, Denmark)

When a fish school swims through the water, every individual consumes a certain amount of oxygen, which means that less will be available for the trailing fish in the school. In 1967 McFarland and Moss reported that the oxygen saturation decreased approximately 30% from the front to the rear of an approximately 150-m long school of mullets swimming in normoxic water. They also observed that the decline in oxygen saturation at the rear resulted in the school disintegrating into smaller separate schools.

Oxygen consumption of swimming fish increases exponentially or as a power function with respect to swimming speed, and hence the decrease in oxygen saturation through the school is related to the swimming speed of the school. A model describing the oxygen saturation in a fish school from front to rear at different swimming speeds will be presented. The model reveals that the school has a maximum length at the optimal swimming speed, and that a very large school cannot swim at slow speeds. Oxygen saturation through a fish school is also influenced by several parameters other than swimming speed, i.e. nearest neighbour distance, water temperature, gill oxygen extraction, gill ventilation capacity, etc. Fish swimming in a school have been shown to have energetic advantages when trailing behind neighbours, resulting in up to 20% energy saving. The effect of this energy saving is that the fish schools can be longer.

A model of oxygen saturation through a fish school will be presented.

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10:55 Monday 2nd July 2012

A2.4
Hydrodynamic advantages of undulatory swimming in schools

Charlotte Hemelrijk (University of Groningen, Netherlands), H. Hildenbrandt (University of Groningen, Netherlands), D. Reid (University of Groningen, Netherlands) and J. Padding (Technical University of Eindhoven, Netherlands)

A number of empirical studies of schools of fish have shown that there are hydrodynamic benefits when fish swim in a school rather than on

their own. It is, however, difficult to understand how fish should configure themselves spatially in order to gain energy advantages, because in a school the wakes of the fish interact. Thus, it is unclear at which locations fish can gain energy from the flow.

The complexity of empirical measurements means that we have studied possible energetic advantages in a computational model of hydrodynamics and fish travelling in a school. Our computational model is based on multiple particle collision dynamics or stochastic rotation dynamics. It generates realistic hydrodynamics from collisions between 30million particles and from the undulating fish (i.e. mullets), such as the reverse von Kàrmàn wake behind a single fish.

Individuals in schools are shown to swim faster and more efficiently than single fish in most of the rigid spatial configurations tested (a square, a diamond, a phalanx and a line). Remarkably, neighbours appear to have a positive effect on efficiency and speed not only when they are to the side but also when they are ahead, despite their backwards jet, and these positive effects appear to strengthen each other. These models generate new predictions to be tested empirically.

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11:45 Monday 2nd July 2012

A2.5
Biomechanics and shape of schools of fish and flocks of birds

Charlotte Hemelrijk (University of Groningen, Netherlands) and H. Hildenbrandt (University of Groningen, Netherlands)

The collective motion of fish in schools and of birds in flocks is supposed to protect against predation. Such an advantage is thought to result, among other things, from the specifics of shape. It is, however, unclear how individuals collectively maintain a specific group shape. Notably, the shape of schools of fish is usually oblong and that of flocks of birds is highly variable.

In the present talk, we study what causes group-shape using new computational techniques based on the processes of self-organization. In our individual-based models of collective motion, individuals coordinate with nearby neighbours. In our models of flocks of birds, individuals are also equipped with simplified rules of the physics of flying.

Patterns of schooling and flocking in our models closely resemble many aspects of the empirical data of schools of fish and flocks of birds, in particular starlings. These models reveal that the specifics of locomotion (swimming *versus* flying) underlie the typical oblong shape of schools of fish and the great variation in the shapes of flocks of birds. We explain how this happens. The model leads to a number of testable predictions.

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13:45 Monday 2nd July 2012

A2.6
Flying in a flock comes at a cost in pigeons

Jim R. Usherwood (Royal Veterinary College [RVC], UK), Steven J. Portugal (RVC, UK), John Lowe (RVC, UK), Kyle Roskilly (RVC, UK) and Alan M. Wilson (RVC, UK)

The highly coordinated movements of flocks of birds are among the most fascinating phenomena to be found in nature. When birds take to the air in a large group, they customarily fly in either a V-formation or a cluster-like swarm. Both flocking approaches can have their benefits. Flying in a V-formation, for example, can result in aerodynamic benefits, thus reducing power requirements, as demonstrated by a reduction in heart rate and wing-beat frequency in pelicans flying in a V-formation. Cluster flocks traditionally offer benefits such as increased detection of predators, while ensuring accurate navigation on migration or to roosts and local feeding areas.

Here we will present research in pigeons that highlights the potentially negative aspects to being in a crowd, and suggests that pigeons flying in a flock gained neither an aerodynamic advantage nor added energy efficiency when flying. We demonstrate that pigeons in a cluster flock

- maintain powered, banked turns like aircraft, imposing dorsal accelerations of up to 2 g, effectively doubling body weight and quadrupling induced power requirements;
- increase flap frequency with increases in all conventional aerodynamic power requirements; and
- increase flap frequency when flying near, particularly behind, other birds.

The increased flap frequency, whether due to direct aerodynamic interactions or requirements for increased stability or control, suggests a considerable energetic cost to flight in a tight cluster flock.

This study was made possible by the use of data from innovative back-mounted GPS and six-degrees-of-freedom inertial sensors custom-built at the Royal Veterinary College, UK.

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14:15 Monday 2nd July 2012

A2.7
Maximizing savings or minimising costs? Cooperative aerodynamics and 'V' formation flight re-examined

Steven J. Portugal (Royal Veterinary College [RVC], UK), Jim R. Usherwood (RVC, UK), Tatjana Y Hubel (RVC, UK), Kyle Roskilly (RVC, UK), Stephen Hailes (University College London, UK), Johannes Fritz (Waldrappteam) and Alan M. Wilson (RVC, UK)

The characteristic 'V' formation flight of birds has fascinated scientists for centuries. One of the main theories that have persisted to explain this distinctive 'V' formation is that birds are attempting to conserve energy by taking advantage of the up-wash vortex fields created by the wings of the birds in front. Aerodynamic theory has subsequently been used to construct predictions of where birds should optimally position themselves in relation to other members within the 'V', to maximize these energy savings. These predictions, however, are based on a fixed-wing principle, very much unlike the scenario of a flapping bird/wing.

Previous investigations into 'V' formation flocking have been largely restricted to theory or the use of photography to examine flock-member positioning. Recent technological advances have now made it possible to explore these factors for extended periods of time in free-flying birds. Using high-frequency sampling GPS and accelerometer units, we will present data from two migratory flights of the critically endangered Waldrapp ibis. This opportunity was made possible by human-led migrations taking place as part of a reintroduction scheme, whereby imprinted young ibis are taught to follow a microlight. These data allow us to explore aspects of 'V' formation flocking relating to distances and angles between individuals, and the interactions these have with flap-frequency. Moreover, it allows comparisons to be made between 'V' formation flight and the recent finding that flying in a cluster flock actually comes at a cost in pigeons, to ascertain whether 'V' formation flight is an energy-saving mechanism.

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14:40 Monday 2nd July 2012

A2.8
Experimental aerodynamic analysis of formation flight in birds

Paul Bomke (University of Groningen, Netherlands) and Eize Stamhuis (University of Groningen, Netherlands)

Larger migratory birds (e.g. geese) often fly in formations that are believed to yield aerodynamic advantages: birds could experience drag reductions by flying in the wing tip up-wash of their companions. Extraordinary energetic savings of up to 70% have been estimated for a flock, but these estimations are based on fixed-wing aerodynamics. Wing motion and three-dimensional flow phenomena occurring during flapping flight have not been taken into account so far. In the present study, an attempt has been made to quantify possible aerodynamic advantages and potential energy savings by mapping both the three-dimensional wake behind a bird model in flapping flight using digital particle image velocimetry, and by studying the interaction of two flapping bird models in wind tunnel setups.

Wing tip up-wash appeared much lower than previously thought but was estimated to still account for drag savings of up to 6%, depending on bird model spacing and wing-phase relation. At only a few relative positions, birds in an oblique line formation can benefit from one another with regard to lift gain, but the advantages measured were very small. When the wings were laterally overlapping, pronounced decreases were observed.

These results indicate a possible but small drag reduction if a fixed wing-beat phase relation is maintained between birds. As this is rarely seen in nature, the proposed energetic advantage of formation flight in migrating birds seems disputable. Our force measurements, moreover, suggest a simple avoidance of the downwash regions from more anterior birds to cause the observed laterally staggered formation.

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15:35 Monday 2nd July 2012

A2.9
Stability analysis of mill formation in a swarm of organisms

Yoshinobu Inada (Tokai University, Japan) and Hikaru Uriu (Tokai University, Japan)

'Mill' is a pattern of formation in which organisms sometimes swarm. It has a ring-shaped structure in which large numbers of individuals move together in a circle. In nature, a mill is formed when individuals at the front of a moving swarm occasionally meet the tail of the swarm and is kept stable for a long period. A famous example is that observed in a school of bigeye trevally (*Caranx sexfasciatus*).

This study focuses on the stability of mill formation and clarifies the factors that affect the stability of mill by using a swarm simulator. The swarm simulator is based on the behaviour of individual in a fish school or a bird flock, simulating three typical behaviours – attraction, parallel orientation and repulsion to other individuals – that are switched on/off depending on the distance between individuals.

The result indicates that the number of neighbours each of the individuals in a swarm can simultaneously interact with significantly affects the stability, enhancing stability with a large number of interacting neighbours. Other factors like the intensity of attraction or parallel orientation did not show much effect on the stability of a mill.

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16:00 Monday 2nd July 2012

A2.10
Leadership, movement decisions, and energetic gain

Sean A. Rands (University of Bristol, UK)

Although social behaviour can bring many benefits to an individual, there are also costs that can be incurred whenever the members of a social group interact. The formation of dominance hierarchies could offer a means of reducing some of the costs of social interaction, but individuals within the hierarchy might end up paying differing costs dependent upon their position within the hierarchy. These differing interaction costs might therefore influence the behaviour of the group, as subordinate individuals might experience very different benefits and costs to dominants when the group is conducting a given behaviour.

Here, a state-dependent dynamic game is described that considers a pair of social foragers where there is a set dominance relationship within the pair. The model considers the case where the subordinate member of the pair pays an interference cost when it and the dominant individual conduct specific pairs of behaviours together. The model demonstrates that if the subordinate individual pays these energetic costs when it interacts with the dominant individual, this has effects upon the behaviour of both subordinate and the dominant individuals.

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16:25 Monday 2nd July 2012

A3 – STRUCTURAL MODULATORS OF MUSCLE CONTRACTION AFFECTING ANIMAL LOCOMOTION AND BEHAVIOUR

A3.1
Structural and functional trade-offs in the design of striated muscle fibres

Robert Josephson (University of California Irvine, California, USA)

Nearly the entire volume of a striated muscle fibre is taken up by but three functional components: myofibrils (the source of mechanical force and power); sarcoplasmic reticulum (SR) and T-tubules (components involved in turning on and off contractile activity); and mitochondria (source of fuel for contraction and its control). The relations between the relative abundance of these components and muscle contractile performance was examined in singing muscles from 11 cicada species, among which the muscle contraction frequency during singing ranged from 40 to 550 Hz. The singing muscles in 10 of the species were synchronous muscles with a muscle action potential for each contraction and the 11 had asynchronous (self-oscillatory) singing muscle.

In the synchronous muscles the twitch duration declined with increasing relative volume of SR and T-tubules (range: 10.2–19.5 % of fibre volume) and with decreasing myofibril area (a measure of diffusion distance and hence diffusion time for calcium into and out of the fibril). As expected from other insects with asynchronous muscles, the cicada with asynchronous singing muscle achieved a high contraction frequency (390 Hz) with a relatively small investment in SR and T-tubules (3% fibre volume). The fractional volume of mitochondria was similar in all muscles (range: 34–42%). There was an inverse correlation between myofibrillar volume and that of SR and T-tubules, indicating that an increase in SR and T-tubules and hence muscle rapidity is at the expense of force and power.

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13:50 Sunday 1st July 2012

A3.2
Biomechanical factors in the neuromuscular control of bipedal locomotion: A compromise between stability and injury avoidance?

Monica Daley (Royal Veterinary College, UK)

To survive in natural environments, animals must achieve robustly stable and agile locomotion over varied terrain. Locomotion is an inherently integrative process, involving complex interactions among neuromuscular and biomechanical factors. It is becoming clear that different neuro-mechanical control strategies likely involve trade-offs among stability, economy and injury avoidance but these trade-offs remain poorly understood. We use a combination of experiments and modelling to understanding how whole-body dynamics, leg posture and muscle-tendon architecture interact to influence the neuromuscular control of locomotion. Experimental measures of *in vivo* muscle contraction and leg biomechanics have revealed that perturbations in leg posture and body velocity at the time of ground contact have a critical influence on the subsequent muscle-contraction dynamics, forces and net muscle work during stance. These studies have highlighted the critical importance of controlling landing conditions and leg posture at the swing-stance transition. The findings suggest that ground birds control landing conditions to minimize peak ground forces during stance across varied terrain. This is achieved through a combination of passive–dynamic and active neuromuscular control. More rapidly stabilizing control strategies are possible, but demand higher peak leg forces and muscle work. Birds opt for a small compromise in stability that may minimize injury risk in uneven terrain.

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14:25 Sunday 1st July 2012

A3.3
Structural modulation of shortening velocity in cephalopod muscle

William M. Kier (University of North Carolina at Chapel Hill, North Carolina, USA)

The modulation of the contractile properties of vertebrate skeletal muscle fibres depends primarily on variation in the isoforms of the contractile proteins of the myofilament lattice with relatively little alteration of the arrangement and dimensions of myofilaments and sarcomeres. In contrast, an integrative analysis of muscle fibres in cephalopods suggests that specialization depends on structural, rather than biochemical changes.

The fast-contracting extensor muscle fibres of the prey capture tentacles of loliginid squid have been compared with the slow-contracting and serially homologous muscle fibres of the arms. Little evidence of differences in contractile protein isoforms between the two fibre types was observed using a variety of SDS polyacrylamide gel electrophoresis techniques and peptide mapping of the myosin heavy chains.

Recently the nucleotide and amino acid sequences of the myosin heavy chain were determined from the arm and tentacle (*Doryteuthis pealeii*) and both express identical myosin isoforms. Thus, modulation of contractile properties in squid muscle fibres does not depend on biochemical change. Instead, specialization in the fast tentacle extensor muscles involves changes in the arrangement and dimensions of the myofilament lattice. The tentacle fibres exhibit cross-striation, unusually short sarcomeres, and thick filaments (~0.8 μm); while those of the arms are obliquely striated with much longer thick filaments (~7.4 μm). The shortening velocity of elements in series is additive, so this structural difference results in a 10-fold higher shortening velocity in the tentacle fibres (V_{max} = 15.4 L0 s^{-1}) compared with the arm fibres (V_{max} = 1.5 L0 s^{-1}).

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14:45 Sunday 1st July 2012

A3.4
Consequences of variations in activation patterns on the mechanical performance of the tentacle strike in squid

Johan L. Van Leeuwen (Wageningen University, Netherlands) and William M. Kier (University of North Carolina, North Carolina, USA)

The two tentacles of squid, which lack hard skeletal support, are used in an aquatic environment to capture prey within 30–100 ms. During the

strike in the squid *Loligo pealei*, the muscular stalks of the tentacles rapidly extend by about 70% in 20–30 ms and the prey is adhered by suckers at the terminal clubs. A forward dynamics model of this system with a series of lumped masses and distributed activation input was developed to predict the extension dynamics of the tentacles (Van Leeuwen, J.L. & Kier, W.M. *Phil. Trans. R. Soc. B* 1997; 352: 551–571). The predictions were in agreement with the kinematic observations from high-speed films (Kier, W.M. & Van Leeuwen, J.L. *J. Exp. Biol*. 1997; 200: 41–53), and also showed that the unusually short myofilaments in the extensor muscles are likely to be an adaptation for high peak extension speed and power output during the strike.

Using the model, we now predict that the efficacy of the tentacular extension is remarkably robust against variations in the activation pattern along the tentacle. A delay along the tentacle of 50% of the extension time has a minor effect on the peak extension velocity of the tentacle compared with a zero delay pattern. This robustness might be due to the mechanical properties of the muscular tissue in the tentacle.

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15:05 Sunday 1st July 2012

A3.5
Radial myofilament motion in *Manduca* muscle modulates mechanics

Tom Daniel (University of Washington, Washington, USA), Nicole George (University of Washington, Washington, USA), David Williams (University of Washington, Washington, USA), Mary Salcedo (University of Washington, Washington, USA) and Tom Irving (Illinois Institute of Technology, Illinois, USA)

As muscles contract, myofilament overlap increases. With constant cell volume, however, the radial distance between thick and thin filaments must increase. Surprisingly, little is known about radial motion of myofilaments in muscles undergoing contraction *in vivo*. Since cross-bridge binding, recruitment and force generation depends upon the distance between the myofilaments, this simple process might contribute to length-dependent force. Additionally, if myofilaments do not expand, there would be movement of intracellular fluid through the filament lattice.

To address this issue we performed work loops on intact *Manduca sexta* flight muscles while simultaneously measuring myofilament spacing, force and length using time-resolved small-angle X-ray diffraction. Muscles subject to a 25 Hz 6% strain sinusoidal length change were stimulated at three different phases. Five X-ray images were taken during each cycle for 100 contraction cycles. Measured lattice spacing:

- strongly reflected activation timing;
- varied considerably during the cycle (2.4%); and
- did not follow the pattern predicted by a constant volume assumption.

The large changes in lattice spacing suggest that models of cross-bridge force generation should include expansion of the lattice. Additionally, cross-bridges can restrict lattice expansion, causing a mismatch between measured and predicted radial motions of myofilaments. This mismatch might require significant fluid movement between subcellular compartments that has not been considered in the mechanics and energetics of force generation by muscle.

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15:40 Sunday 1st July 2012

A3.6
Cross-bridge kinetics slow with random myosin loss from thick filaments

Bertrand C.W. Tanner (University of Vermont [UoV], Vermont, USA), Mark S. Miller (UoV, USA), Michael J. Toth (UoV, Vermont, USA) and Bradley M. Palmer (UoV, Vermont, USA)

Myosin is lost from skeletal muscle fibres with ageing, disuse and disease, potentially decreasing whole-muscle contractile performance. Measurements from our laboratories show loss of myosin content (17–36%) and prolonged cross-bridge attachment time, ton (14–19%), in chronic heart failure patients, compared to healthy, activity-matched controls. We observed no differences in myofibrillar area, A-band length or thick-to-thin filament ratio between patients and control individuals, suggesting that myosin loss occurred randomly along thick filaments.

To investigate how thick filament structural changes influence ensemble cross-bridge behaviour, we simulated myosin loss using a computational model of the half-sarcomere. Randomly reducing myosin content (10–50%) slowed cross-bridge cycling and linearly increased ton (5–40%), in agreement with our measurements from heart failure patients. Reducing myosin content from the free end of thick filaments slightly decreased ton (10%) at 20–30% myosin loss and prolonged ton (15%) at 50% myosin loss. Maximal steady-state tension decreased less when myosin loss occurred randomly than when it occurred from the free end of thick filaments (5–40% *versus* 15–60%, respectively).

These simulations predict that structural variations in thick filaments directly influence ensemble cross-bridge behaviour by varying the load borne by individual cross-bridges, thus altering strain-dependent kinetics at the molecular level and force production at the cellular level. These findings further support the idea that myosin loss from skeletal muscles occurs randomly along thick filaments with heart failure and provides a means for investigating the effects of myosin loss in other diseases and due to disuse and ageing.

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16:00 Sunday 1st July 2012

A3.7
The effect of thermal acclimation on isometric force generation and cross-bridge kinetics in trout cardiac trabeculae

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

In most endothermic species, a decrease in body temperature can lead to heart failure. The primary cause of this is a decrease in Ca^{2+} sensitivity of the cardiac myocytes. Interestingly, the heart of the rainbow trout remains functional at 4ºC. Previous work has shown that cold acclimation results in cardiac hypertrophy and that this hypertrophic response involves an increase in muscle mass and connective tissue within the ventricle. Furthermore, cold acclimation caused a 50% increase in the maximal rate of the actomyosin ATPase. Recently we examined how these changes in actomyosin ATPase activity translate into rates of force development and the ability of isolated cardiac trabeculae to generate force in response to Ca^{2+}.

We found that there was no difference in the maximal isometric force generated but that cold acclimated samples were more sensitive to Ca^{2+} (pCa_{50} = 5.69 ±0.02) compared to control and warm acclimated samples (pCa_{50} = 5.59 ±0.02 and 5.61 ±0.01, respectively) when tested at 15ºC with a sarcomere length of 2.2 µm. Interestingly, when cold-acclimated and control trabeculae were tested at 8ºC, there was no difference in the force-generating capacity over the physiological range of activation, but the rate of cross-bridge cycling of the cold-acclimated samples over this range was ~20–40% less than controls. This suggests that cold-acclimated trout cardiac muscle is able to generate more force per cross-bridge than control cardiac muscle. A model to explain this phenomenon is currently being developed.

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16:20 Sunday 1st July 2012

A3.8
Separate evolution of flightin domains reflect functional duality in flight and courtship in *Drosophila*

Jim O Vigoreaux (University of Vermont, Vermont, USA), Hien Vu (University of Vermont, Vermont, USA) and Samya Chakravorty (University of Vermont, Vermont, USA)

In *Drosophila*, the indirect flight muscles (IFMs) are the major power producing muscles for flight. Additionally, the IFMs are neurally activated during courtship song but their role in acoustic production has not been defined. We investigated the effect of mutations in flightin, an IFM-specific myosin-binding protein that is essential for normal thick filament structure and integrity, on IFM-produced courtship behaviours and flight.

Among drosophilids, the N-terminal flightin sequence is highly variable (~15% identity) compared to the rest of the protein (>70% identity). Transgenic *D. melanogaster* strains expressing a truncated flightin that is missing 62 N-terminal amino acids ($fln^{\Delta 2\text{-}63}$) have normal myofibrillar structure, but are flight compromised compared to control wild-type rescued null flies (fln^{+}; flight indices of 3.12 ±0.34 and 4.2 ±0.36, respectively). Despite a normal wing beat frequency (198 ±4 Hz), power output in skinned IFM fibres is reduced for $fln^{\Delta\ 2\text{-}63}$ (62 ±6 *versus* 93 ±11 W m^{-3}). Courtship song analysis showed that $fln^{\Delta\ 2\text{-}63}$ males sing with longer interpulse intervals (IPI, 60 ±6 ms *versus* 40 ±2 ms), higher sine song frequencies (220 ±3 Hz *versus* 149 ±10 Hz), and similar intrapulse frequencies compared to fln^{+} males. Mating competition assays show female preference for fln^{+} males over $fln^{\Delta 2\text{-}63}$ males, indicating that changes in song quality adversely affect female choice.

We conclude that the variability in N-terminal flightin sequences reflects a primary role in sexually-selected courtship behaviour. Thus, it appears that flightin influences flight and courtship through separate protein domains that are under distinct evolutionary selection.

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16:40 Sunday 1st July 2012

A3.9
Analysis of the projectin PEVK domain, a *Drosophila* titin homologue, for flight and body muscle function

Wouter Koolhaas (Max Planck Institute of Biochemistry, Germany), Maria Spletter (Max Planck Institute of Biochemistry, Germany) and Frank Schnorrer (Max Planck Institute of Biochemistry, Germany)

The asynchronous indirect flight muscles of insects are highly specialized powerful muscles, which empower rapid thorax and wing oscillations to enable flight. Asynchronous muscle contractions require calcium; however, the high oscillation frequency is achieved by a stretch activation mechanism of the muscle. *Drosophila* beats its wings at 200 Hz and during each contraction cycle flight muscles shorten only 3–4%. This mechanism requires a high passive stiffness of the flight muscles compared to other body muscles.

It is hypothesized that vertebrate titin and its fly homologues projectin, kettin and sallimus are the main contributors to passive stiffness. In particular, the length of the elastic PEVK domain present in these proteins determines their flexibility upon stretch *in vitro*. We identified different splice variants of projectin in flight muscle compared to leg muscles that interestingly differ in the length of their PEVK domains. The stiffer flight muscles possess a shorter PEVK domain.

We plan to manipulate the length of the projectin PEVK domain *in vitro* and test the functional and mechanical consequences of this manipulation *in vivo* by assaying flight ability and by measuring flight muscle fibre stiffness. This should lead to an *in vivo* assessment of the contribution of the PEVK domain to passive stiffness and flight muscle function. As this domain is conserved to vertebrates and differs in length between body muscle and stiffer heart muscle, the conclusions obtained should be of general relevance for muscle biology.

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Poster Session – Saturday 30th June 2012

A4 – TOWARDS A NEW SYNTHESIS OF THE PHYSIOLOGY AND EVOLUTION OF DIVING

A4.1
Multilevel integration of adaptations for maximizing aerobic dive duration in marine mammals: From biochemistry to behaviour

Randall W. Davis (Texas A&M University, Texas, USA)

Marine mammals exhibit a suite of adaptations that enable them to remain submerged for much of their lives while maintaining aerobic metabolism. This phylogenetically-diverse group of mammals representing three taxonomic orders (Carnivores, Cetaceans and Sirenians) evolved from terrestrial mammals that never reacquired the ability to breathe water. As a result, they must divide their time between two critical resources: food at depth and oxygen at the surface. These two resources and the long (25million to 55million years), convergent evolution of adaptations that maximize the efficient use of time and energy in the aquatic environment have influenced every aspect of marine mammals, from their biochemistry to behaviour.

Here, I review some of the major adaptations that are integrated around the need for maintaining convective oxygen transport, aerobic metabolism, physiological homeostasis, and the efficient use of time and energy. These adaptations include:

- the molecular structure and oxygen affinity of myoglobin and haemoglobin;
- aerobic and beta-oxidation enzyme activities;
- mitochondrial volume density in muscles and other organs;
- oxygen stores in the blood and muscle;
- fuel homeostasis;
- organ function and physiological homeostasis;
- hydrodynamic body morphology and cost-efficient modes of locomotion; and
- foraging strategies that minimize cost and maximize energy intake.

The multi-level integration of these adaptations has enabled marine mammals to exploit all of the world's oceans from surface waters to depths of over 2,000 m, one of the largest ecosystems on earth.

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10:30 Sunday 1st July 2012

A4.2
Evolution of marine endotherms

Mark D. Uhen (George Mason University, Washington, USA)

Mammalian and avian lineages have repeatedly evolved to live in aquatic environments over their evolutionary histories. Some have evolved semi-aquatic life habits, being tied to the terrestrial environment for breeding, birth and perhaps raising of young. Several avian lineages have lost the power of flight while adapting to the marine realm. The mammalian orders Cetacea and Sirenia have become fully aquatic.

This review includes an introduction to the evolutionary history of each group of marine endotherms based on studies using morphologic, stratigraphic and molecular data. Most of these aquatic lineages have features in common, such as a fusiform body, flipper-shaped forelimbs and increased body fat. Many other systems, such as senses, locomotion, breathing, feeding and reproduction, are adapted to the aquatic environment in various ways.

Results show that the aquatic characteristics evolved in mosaic patterns and the different morphological solutions to aquatic conditions were achieved separately in each of these groups. Among aquatic avians, changes in the appendicular skeletons indicate an emphasis on wing-propelled or foot-propelled diving. Only after the loss of flight do changes in bone histology to enhance diving abilities occur. Among aquatic mammals, only the fully aquatic Cetacea and Sirenia abandon limb-based locomotion for axis-based locomotion. Mammalian carnivores exhibit a simplification and/or reduction of the dentition related to feeding on aquatic prey. The earliest representatives of these clades indicate that they were feeding in the water, suggesting that feeding ecology was a key factor in the evolution of marine endotherms.

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11:20 Sunday 1st July 2012

A4.3
The advent of diving behaviour of aquatic mammals: Association of hydrodynamics, swimming mode and thermoregulation in relation to energetics

Frank E. Fish (West Chester University, West Chester, USA)

The secondary invasion of mammals into the aquatic environment required morphological changes that were associated with the hydrodynamic requirements for reduced drag and increased thrust production, which impacted the energetics of locomotion. The transition from fully terrestrial locomotion to fully aquatic locomotion produced intermediate forms that were semi-aquatic and semi-terrestrial. The duality of such intermediate forms would incur high energetic demands both on land and in water due to the change to a more robust musculoskeletal arrangement.

Larger and stockier skeletal elements, particularly in the appendages, were associated with postural control in water, and the larger force production needed for drag-based propulsion and the increased resistance from the production of surface waves in the water. The development of diving mitigated the hydrodynamic drag of surface swimming and opened up new sources of food. Enhanced swimming performance (i.e., speed, manoeuvrability and energetic efficiency) while submerged required a shift to lift-based propulsion systems, utilizing oscillating wing-like appendages (e.g. flippers and flukes).

With deeper dives and longer periods immersed in water, thermoregulation and the demands of buoyancy control dictated the replacement of air-trapping fur with blubber. These steps culminated in aquatic forms (e.g. cetaceans, pinnipeds and sirenians), which have taken morphological evolution to the extreme and now manage their energetics through behavioural and physiological mechanisms.

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13:10 Sunday 1st July 2012

A4.4
Dive performance and aquatic thermoregulation of the world's smallest mammalian diver, the American water shrew (*Sorex palustris*)

Roman W. Gusztak (University of Manitoba, Winnipeg, Canada), Robert A. MacArthur (University of Manitoba, Winnipeg, Canada) and Kevin L. Campbell (University of Manitoba, Winnipeg, Canada)

The 12–17 g American water shrew, *Sorex palustris*, is the world's smallest mammalian diver. Allometry predicts this species to have the smallest total body oxygen storage capacity, highest diving metabolic rate, lowest skeletal muscle buffering capacity and lowest glycolytic capability of any endothermic diver. Despite these constraints, the calculated aerobic dive limit (cADL; 14.2 sec) of water shrews is close to that predicted by allometry for larger-bodied divers. Wild-caught water shrews generally adhered to predicted ADLs while submerged, with only 2.3–3.1% of dives exceeding their cADL. The mean voluntary dive time of water shrews was 5.1 ±0.1 sec (N=25, n=1,584), with a mean maximum dive time of 10.3 ±0.4 sec, and a maximum dive time of 23.7 sec. Dive trials completed in 3, 10, 20 and 30°C water showed that water temperature significantly affected dive duration, duration of the longest dive and total time in water, but not dive frequency.

Interestingly, our results do not support the 'adaptive hypothermia' hypothesis. Shrews regularly used behavioural thermoregulation to defend against immersion hypothermia, with <5% of dives occurring at body temperatures <37.3ºC. Similarly, radio-telemetered shrews housed in a simulated riparian environment for 24-hour trials (water temperature = 3°C) consistently elevated core body temperature by 1.0–1.5ºC in the few minutes prior to initiating aquatic foraging bouts, and ended these bouts when body temperature was still above normal resting levels (~37.8°C). We suggest this observed pre-dive 'hyperthermia' aids to heighten the impressive sensory abilities of this diminutive predator while submerged.

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14:00 Sunday 1st July 2012

A4.5
Evolution of mammalian diving capacity and the aquatic ancestry of elephants

Michael Berenbrink (Liverpool University, UK), Scott Mirceta (University of Manitoba, Winnipeg, Canada and Liverpool University, UK), Anthony Signore (University of Manitoba, Winnipeg, Canada) and Kevin Campbell (University of Manitoba, Winnipeg, Canada)

The ability of elite mammalian breath-hold divers to actively forage for extended periods in the depths of the oceans is among the most celebrated of physiological adaptations. The skeletal modifications for energy-efficient locomotion that evolved during the transition from quadruped terrestrial ancestors to increasingly aquatic mammals are well documented (Uhen, *Anat. Rec*. 2007; 290: 514–522.). Stable isotope composition of fossil tooth enamel can even indicate whether a species spent appreciable time in an aquatic environment (Liu *et al*., *PNAS* 2008; 105: 5786–5791). Despite this, the evolution of physiological diving capacity in ancestral mammals has remained completely unknown.

Here, we show that skeletal muscle content of oxygen-storing myoglobin accurately predicts body mass corrected maximal dive time across multiple lineages of extant mammalian divers. We identify a strong molecular signature of maximum muscle myoglobin content in the amino acid sequences of mammalian myoglobins. Using ancestral sequence reconstruction, we trace the evolution of muscle oxygen stores, and thereby diving capacity, in a mammalian phylogeny.

When applied to *Afrotheria*, this surprisingly suggests a period of strong increase of maximal tissue myoglobin content in the elephant lineage after its divergence from the sea cows and a marked decrease prior to the radiation of recent elephant species. These results support an aquatic, even diving, period in the ancestry of modern elephants, a claim that has been debated for more than 100 years and has recently been supported by stable isotope analyses of fossil tooth enamel from elephant ancestors.

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14:30 Sunday 1st July 2012

A4.6
Marine mammal neurology: Divergent molecular strategies in the diving brain

Marco Schneuer (Institute of Zoology and Zoological Museum [IZZM], University of Hamburg, Germany), Nicole U. Czech-Damal (IZZM, University of Hamburg, Germany), Lars P. Folkow (University of Tromsø, Norway), Arnoldus S. Blix (University of Tromsø, Norway), Ursula Siebert (Research and Technology Centre Büsum, Germany) and Thorsten Burmester (IZZM, University of Hamburg, Germany)

Many physiological adaptations in diving mammals have been reported but currently little is known about how the brains of whales and seals sustain the high demands of metabolic energy and thus O_2 when submerged. Our studies in deep diving hooded seals (*Cystophora cristata*) and harp seals (*Pagophilus groenlandicus*) revealed a shift of neuroglobin, a respiratory protein involved in neuronal hypoxia tolerance and the oxidative energy metabolism from neurons to astrocytes. From this, we assume that neurons of deep diving seals largely rely on anaerobiosis.

In the shallow diving harbour seal (*Phoca vitulina*) as well as in the whale brain, however, neuroglobin resides in neurons similarly to terrestrial mammals, although we observed higher neuroglobin mRNA expression levels in harbour porpoise (*Phocoena phocoena*) and minke whale (*Balaenoptera acutorostrata*) than in cattle (*Bos taurus*), indicating an enhanced oxygen supply in the whale brain. The expression patterns of antioxidant enzymes in whales and seals also differ: compared to their terrestrial relatives in the seal brains levels of superoxide dismutase 2 are significantly enhanced, whereas there are no differences between whales and cattle.

To identify the cellular mechanisms of the metabolic shift in the seal brain, we investigated the distribution of enzymes and transporters of energy metabolism in deep diving seals. Our results indicate that whales and seals developed divergent molecular mechanisms to cope with hypoxic challenges in their brains. This is likely due to an independent evolution and adaptation to a marine lifestyle.

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15:20 Sunday 1st July 2012

A4.7
Convergent molecular evolution of myoglobin in avian and mammalian divers

Scott Mirceta (University of Manitoba, Winnipeg, Canada), Kevin L. Campbell (University of Manitoba, Winnipeg, Canada) and Michael Berenbrink (University of Liverpool, UK)

Deprivation of external oxygen (O_2) supply is a central problem for air-breathing vertebrates while breath-hold diving. Despite this, remarkable feats of underwater submersion have been observed, with some mammals routinely diving for over an hour. It has been suggested that muscle O_2 stores are key determinants of dive duration, with the amount of O_2 carried dictated by the muscle myoglobin (Mb) concentration. Not surprisingly, high Mb concentrations are thus a consistent hallmark of accomplished avian and mammalian divers.

Recently we showed that a molecular signature of increased Mb net charge is present in all 10 independent lineages of diving mammals examined, and that Mb net charge, as calculated from protein sequence,

is significantly correlated with Mb concentration and dive duration. Furthermore, ancestral Mb sequence reconstruction shows striking evolution of high Mb net charge across mammalian diving lineages.

Here we present an analysis of 57 avian Mb protein sequences and show that Mb net charge is generally higher in birds compared to mammals, with diving species predictably exhibiting the highest charges among birds. As with mammals, muscle Mb concentrations are also higher in diving birds compared to non-divers, with a strong correlation between net protein charge, Mb concentration and dive duration. Our findings thus indicate that a remarkable adaptive convergent evolution of the myoglobin protein contributed to the impressive dive performance of diving birds and mammals.

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15:45 Sunday 1st July 2012

A4.8
Aerobic and anaerobic muscle energy stores in diving penguins

Cassondra L. Williams (University of California Irvine, California, USA), Katsufumi Sato (University of Japan, Japan), Kozue Shiomi (University of Japan, Japan) and Paul J. Ponganis (University of California San Diego, California, USA)

During diving, birds and mammals must balance the energy demands of working muscle with the need to conserve oxygen for vital organs. Bradycardia and extreme peripheral vasoconstriction potentially isolate working muscle from the circulation in diving animals. During complete ischaemia, the amount of ATP produced is dependent on the magnitude of the myoglobin oxygen (O_2) store and the concentrations of glycogen (Gly) and phosphocreatine (PCr).

Gly and PCr concentrations were determined in the pectoralis–supracoracoideus complex, the primary locomotory muscle of emperor penguin. Gly and PCr concentrations (54.6 and 20.8 mmol kg^{-1}, respectively) were similar to concentrations in non-diving animals. Depletion of the muscle O_2 store and the Gly and PCr stores under ischaemic conditions was modelled using a muscle metabolic rate (12.4 mL O_2 kg $muscle^{-1}$ min^{-1}) determined in freely-diving emperor penguins wearing a custom-designed near-infrared spectrophotometer backpack recorder.

Although Gly and PCr concentrations were not elevated, the model demonstrated that Gly and PCr were a significant anaerobic energy store and played a vital role in the emperor penguin's ability to dive for extraordinarily long durations.

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16:10 Sunday 1st July 2012

A4.9
Diving behaviour and energetics of Arctic great cormorants

Patrick J. Butler (University of Birmingham, UK)

Populations of great cormorants, *Phalacrocorax carbo*, range from equatorial Africa to the high Arctic. Their plumage is partially wettable, however, and modelling has indicated that those living in the high Arctic have a high metabolic rate and require about 75% more fish per day than those wintering in Scotland. Frequency and depth of diving during winter are greater, as the light level increases in the morning and during the middle of the day and is lower during night-time. This suggests that these birds are visually-guided predators, even though their visual acuity in water is poor.

Despite the fact that these birds live in cold Arctic water and have feathers with poor insulation properties, their daily rate of energy expenditure during winter, as estimated by the heart rate method from birds freely diving off the west coast of Greenland, is within the range of those for other species of birds. This is related to the fact that they spend relatively little time submerged per day, between 0.5 and 1.0 hour, and are very efficient foragers, catching on average 19 g $fish.min^{-1}$ when they are submerged. While impressive, this is not as high as predicted from laboratory experiments and modelling studies.

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16:35 Sunday 1st July 2012

A4.10
Blood oxygen transport and depletion: The key of consummate divers

Jessica U. Meir (University of British Columbia, British Columbia, Canada)

Bio-logging instruments capable of continuously measuring PO_2 in the blood while diving have revealed exceptional hypoxemic tolerance and highly efficient O_2 utilization in the consummate avian and pinniped divers, the emperor penguin and elephant seal. Characterization of the O_2–haemoglobin dissociation curves of these species has uncovered biochemical adaptations relevant to dive capacity. Application of these curves to blood PO_2 profiles obtained with backpack recorders during diving demonstrates differing O_2 management strategies between these remarkable divers.

In emperor penguins, arterial O_2 saturation (SO_2) remained near 100% for much of the dive, preserving high O_2 content in the arterial system. Arterial SO_2 did not decrease significantly until the final ascent, consistent with declining ambient pressure, air sac and arterial PO_2. These profiles demonstrate the significance of the respiratory oxygen store and the emperor penguin's high-affinity haemoglobin. In contrast, although arterial SO_2 rises and peaks near 100% in the initial dive phase of elephant seals, these values subsequently decrease rapidly, reaching as low as 6–8% and demonstrating exceptional hypoxemic tolerance. Venous SO_2 profiles revealed highly efficient, near-complete utilization of venous O_2 stores during the dives of both species.

These findings reflect differences in the magnitude of respiratory O_2 stores and maintenance of gas exchange during diving between this bird and mammal, revealing attributes that undoubtedly contribute to their extraordinary dives. These results have also recently been reinterpreted with dive type, exposing a novel perspective on the link between physiology and behaviour in the elephant seal.

Funding: National Science Foundation.

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09:00 Monday 2nd July 2012

A4.11
Physiological development of juvenile marine mammals: Implications for the transition to independent foraging

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All young pinnipeds are limited in their diving ability by immature tissue oxygen reserves. In many species, the significantly reduced myoglobin loads observed in skeletal and cardiac muscles of neonatal pups (12–33% of adult values) are accompanied by reduced acid buffering ability (51–84% of adult values), lower metabolically-scaled aerobic (citrate synthase: 9.2–31.9% and β-hydroxyacyl-CoA dehydrogenase:

11.4–62.4%) and anaerobic enzyme activity (lactate dehydrogenase: 8.7–22.0% of adult values). These findings suggest that, relative to adults, pup muscles can not generate as much ATP aerobically or sustain glycolytic ATP production for as long.

In addition, the muscles of young pups have a larger proportion of the fast myosin isoform, MHC-IIA, and lower proportion of MHC-IID/X and the slow isoform MHC-I than those of adults, with pups of some species also expressing embryonic (5–17%) and neonatal (<10%) MHC isoforms. The presence of these isoforms suggests that young pup muscles might have slower contractile speeds and less force-generating capacity. Captive trials during which hooded seal pups were allowed or prevented access to water during post-weaning fast indicate that activity has little impact on the developmental pattern, while developmental shifts in iron stores suggest that early foraging might be essential to obtain the nutrients necessary for final maturation.

In combination, these findings indicate that the precocial appearance of young pups belies the fact that their muscles are likely unable to support extensive underwater activity due to biochemical and structural immaturity, and suggests that early foraging activity is both limited by, and necessary for, final maturation.

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09:30 Monday 2nd July 2012

A4.12
Diving into old age: Senescence in swimming seabirds

Kyle H. Elliott (University of Manitoba, Winnipeg, Canada), Tony Gaston (Environment Canada, Quebec, Canada), Yan Ropert-Coudert (CNRS, France) and James Hare (University of Manitoba, Winnipeg, Canada)

There is now overwhelming evidence that wild animals show actuarial senescence – an increase in mortality rate – with age. Nonetheless, it is unclear what type of physiological decay is responsible for actuarial senescence in wild animals, especially in wild birds that often maintain physiological condition well into old age. Animals undergoing extreme activities, such as diving to great depths, might be expected to show the clearest vulnerabilities to physiological senescence. Recent measurements have shown alterations in the muscle composition of old, diving mammals.

To examine the potential for physiological senescence in diving birds (thick-billed murre, *Uria lomvia*), we examined fine-scale behaviours that should be closely linked to both muscle condition and survival. We measured haematocrit (N=104) and blood volume (N=12) to provide an index of potential oxygen stores and resting metabolic rate as an index of minimum diving metabolic rate (N=43) and equipped birds with time-depth-temperature loggers (N=234) and accelerometers (N=61).

Oxygen stores and resting metabolic rate decreased linearly with age, apparently cancelling each other out, as dive depth, dive duration, surface pause duration, descent rate, wing-beat frequency (underwater and in air) and dynamic body acceleration did not change with age. We concluded that long-lived diving seabirds maintain physiological condition into old age, in support of a catastrophic mortality model of seabird senescence: mortality appeared to occur through the sudden onset of symptoms rather than via detectable, gradual change in physiological condition with advancing age.

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10:20 Monday 2nd July 2012

A4.13
Diving lung volume of Cuvier's beaked whale (*Ziphius cavirostris*) and Northern bottlenose whale (*Hyperoodon ampullatus*) measured using biomechanical data

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Diving lung volume (DLV) influences both oxygen-loading for aerobic metabolism and nitrogen-loading, which increases the risk of decompression syndromes – an important trade-off for deep-diving specialists like beaked whales. We used a hydrodynamic model to estimate the non-gas body density and DLV of two beaked whale species (*Ziphius cavirostris* and *Hyperoodon ampullatus*) using high-resolution measurements of animal depth, three-axis acceleration, and speed during numerous glides made in both descent and ascent phases. The body density of both species measured from glides >200 m deep ranged from 1,029.8 ±0.1 to 1,031.8 ±0.1 kg/m^3, indicating negative buoyancy in seawater and little variation between individuals in our sample. Pooling all glides from deep dives (>1,000 m) by individuals, the DLV of three *Hyperoodon* ranged from 15.5 ±6.2 ml/kg to 22.6 ±1.8 ml/kg and DLV for the single *Ziphius* was 20.8 ±2.5 ml/kg. DLV during dives to shallower depths of 100–500 m were 21.7 ±4.6 ml/kg for the *Ziphius* and 10–12 ml/kg for a single dive to 113 m for *Hyperoodon*. The diving pattern of the *Ziphius* had striking day–night differences, with greatly reduced surface time during the night. DLV also differed strongly between daytime (23.1 ±3.2 ml/kg) and night time dives (9.4 ±5.7 ml/kg). These first measurements of beaked whale DLV confirm anatomical predictions of relatively smaller lung stores for deep compared to shallow diving cetaceans. The observed case of facultative reduction of DLV during periods of intense diving supports the conclusion that nitrogen risks outweigh oxygen benefits for extreme divers like the *Ziphiidae*.

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10:50 Monday 2nd July 2012

A4.14
Blood oxygen depletion in diving California sea lions: The role of the lung oxygen store

Birgitte I. McDonald (Scripps Institution of Oceanography [SIO], University of California San Diego, California, USA) and Paul J. Ponganis (SIO, University of California San Diego, California, USA)

The rate and magnitude of O_2 store depletion is critical to the dive performance and foraging ecology of breath-hold divers. We investigated blood O_2 depletion with a backpack recorder for the partial pressure of O_2 in nine California sea lions during maternal foraging trips. Arterial and venous minimum haemoglobin saturations (SO_2) were routinely >50% and variable during routine shallow dives. In isolated deep dives greater than four minutes in duration, minimum venous SO_2s routinely reached values <10%, and minimum arterial SO_2s were occasionally <20%, indicative of exceptional hypoxemic tolerance. Minimum venous SO_2s were sometimes as low as 1%, consistent with near-complete venous blood O_2 depletion. In a six-minute dive, such depletion of venous blood O_2 contributes 2.7 ml O_2 kg^{-1} min^{-1} to dive metabolic rate. During serial deep dive bouts, both arterial and venous SO_2 increased during ascent, suggesting re-expansion of collapsed lungs and resumption of gas exchange during the 'ascent tachycardia'. Arterial SO_2 depletion patterns of serial deep dives differed from shallow dives and isolated deep dives with abrupt declines and increases at about 200 m depth during descent and ascent, respectively. We suggest this is due to interruption of gas exchange at depth and that an O_2 reservoir in the lungs serves to supplement blood O_2 levels during ascent. Although California sea lions have extreme hypoxemic tolerance and blood O_2 contributes significantly to metabolic rate during a dive, the lung O_2 store might play a greater role than previously suspected in serial deep dives.

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11:20 Monday 2nd July 2012

A4.15

Seasonal variation in oxygen stores attributed to diving behaviour, environmental temperature and pregnancy in the California sea lion

Stella Villegas-Amtmann (University of California Santa Cruz, California, USA), Shannon Atkinson (University of Alaska Fairbanks, Alaska), Alberto Paras-Garcia (Africam Safari, Puebla Zoo, Mexico) and Daniel P. Costa (University of California Santa Cruz, California, USA)

Survival depends on an animal's ability to find and acquire prey. In diving vertebrates, this ability is directly related to their physiological capability (e.g. oxygen stores). We studied the seasonal variation in oxygen stores, body temperature and body condition in California sea lions (*Zalophus californianus*) as a function of seasonal variation in temperature, primary productivity, diving behaviour and reproductive stage.

During summer, blood oxygen stores (haemoglobin concentration [Hb]: 17.5 ±1.9 g/dL, blood volume [B_v]: 136.6 ±28.9 ml/kg, Plasma volume [P_v]: 69.1 ±11.4 ml/kg) were significantly greater and muscle oxygen stores (myoglobin concentration [Mb]: 3.9 ±0.6 g/100g) were significantly lower than in winter (Hb: 15.9 ±1.0 g/dL, p=0.03; B_v: 108.6 ±20.8 ml/kg, p=0.02; P_v: 54.4 ±10.9 ml/kg, p<0.00; Mb: 4.5 ±0.7 g/100 g, p = 0.02). Total oxygen stores, body condition and body temperature did not change between seasons but variations in body temperature were greater during summer.

Changes in oxygen stores are partly attributed to diving behaviour, temperature and pregnancy that could increase oxygen consumption. Blood and muscle oxygen stores appear to be influenced by reproductive state. Blood oxygen stores are more likely influenced by diving behaviour and temperature than muscle oxygen stores.

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11:40 Monday 2nd July 2012

A4.16

Diving capacity of Steller sea lions: The role of oxygen and carbon dioxide

Carling D. Gerlinsky (University of British Columbia [UBC], British Columbia, Canada), David A.S. Rosen (UBC, British Columbia, Canada) and Andrew W. Trites (UBC, British Columbia Canada)

The diving ability of marine mammals is typically defined and limited by the depletion of oxygen stores. Rising carbon CO_2 in the blood also affect respiration in mammals, however, and must also play a role in the physiological control of diving. Surprisingly, few studies have investigated how diving capacity is affected by the accumulation of CO_2 during diving and the subsequent ability to offload this by-product. Our study examined the effects of hypoxia (low O_2) and hypercapnia (high CO_2) on the dive patterns, breathing frequency, metabolic rate and recovery time of Steller sea lions.

We created hypoxic and hypercapnic conditions by altering the composition of inspired gas while animals (n=4) dove voluntarily to 40 m. Rates of oxygen consumption (V_{O2}) and carbon dioxide production (V_{CO2}) were measured using flow-through respirometry. We found that pre- and post-dive ventilation rates increased when inspired CO_2 levels reached 2% in diving animals, although this did not alter dive patterns (dive durations, inter-dive surface times). Post-dive recovery of V_{CO2} took significantly longer than V_{O2} in nearly all dives, however, and hypoxia and hypercapnia further increased recovery time for V_{O2} and V_{CO2}, respectively.

Our study demonstrates the importance of both oxygen store depletion and CO_2 accumulation in limiting diving ability. While oxygen stores limit dive time, CO_2 plays a significant role in the recovery time between dives.

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12:00 Monday 2nd July 2012

A4.17

The comparative biology of diving in European diving beetles: Towards a better understanding of the allometry of diving in ectotherms and endotherms divers

Piero Calosi (Plymouth University, UK), Wilco C.E.P Verberk (Plymouth University, UK), Francois Brischoux (CEBC-CNRS, France), John I. Spicer (Plymouth University, UK), Theodore Garland Jr. (University of California Riverside, California, USA) and David T. Bilton (Plymouth University, UK)

Surfacing behaviour is fundamental in the ecology of aquatic air-breathing organisms. To date, the evolutionary ecology of diving has only been well characterized in vertebrates. Here we report investigations of the diving behaviour of 25 taxa of European dytiscid beetles from the genus *Deronectes* and *Ilybius*.

Beetles were acclimated at two temperatures to help with elucidating how species of the two genera meet increased oxygen demand at higher temperatures. We show that species from different genera have evolved fundamentally different diving responses, which represents an example of 'multiple solutions' in diving. Furthermore, we show that widespread northern species possess higher diving performances than their geographically-restricted southern relatives, our findings suggesting that diving performance underpins geographic range expansion. Finally, the allometric slopes of dive responses in these dytiscids appears similar to those of vertebrate ectotherms, and it appears that allometry of diving in vertebrate ectotherms is closer to that of invertebrates than to that of vertebrate endotherms.

These lines of evidence support the notion that metabolic mode (ectothermy *versus* endothermy) has helped shap the evolution of diving performance.

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12:20 Monday 2nd July 2012

A5 – PHYSIOLOGY OF THE MULTI-FUNCTIONAL GUT

A5.1
PEG provides a particularly profitable probe for perusing permeability

Chris M. Wood (University of Miami, Florida, USA), Carol Bucking (McMaster University, Ontario, Canada) and Martin Grosell (University of Miami, Florida, USA)

Radiolabelled oligomers of polyethylene glycol (PEG) of three different molecular sizes (PEG-400, PEG-900 and PEG-4,000) were used to study permeability relationships *in vitro* in gut sac preparations from the euryhaline killifish, *Fundulus heteroclitus*.

All three PEG molecules were absorbed, with permeability declining as a linear function of increasing hydrodynamic radius. There were no changes in PEG permeability for any size oligomer, despite up to four-fold variations in absorptive water flux associated with feeding and fasting. PEG permeability and absorptive water flux were slightly higher in freshwater *versus* seawater preparations. Experiments using osmotic clamping, reversed gradients and blockers of paracellular permeability (TAP) and aquaporins ($HgCl_2$) indicated entirely separate pathways for PEG (paracellular) *versus* water flux (transcellular) across the intestine.

Labelling of the diet with [^{3}H]PEG-4,000 also proved useful in studies of the uptake and fate of dietary sodium (^{22}Na) and chloride (^{36}Cl) in killifish *in vivo*. Sharp increases in [^{3}H]PEG-4,000 appearance in the water provided a clear definition of defecation events, allowing separation of the systemic efflux of absorbed ions via gills and kidney from their potential efflux into the water via defecation. Absorption of both ^{22}Na and ^{36}Cl from the diet was essentially complete by nine hours prior to the start of defecation, but the extent of systemic efflux *versus* retention varied with salinity, NaCl content of the food and ion (Na *versus* Cl).

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11:00 Friday 29th June 2012

A5.2
The marine teleost intestine: a very multi-functional organ

Rod W. Wilson (University of Exeter, UK)

Dehydration is perhaps the greatest day-to-day physiological challenge faced by marine teleost fish. This is why they ingest seawater at such high rates to maintain osmotic homeostasis. Water absorption occurs in the intestine and is driven by a combination of:

- co-absorption of NaCl;
- Cl^-/HCO_3^- exchange resulting in net bicarbonate secretion;
- precipitation of ingested Ca^{2+} as $CaCO_3$; and
- the advantageously low osmotic coefficient of unabsorbed $MgSO_4$ that accumulates along the gut.

Very high rates of intestinal HCO_3^- base secretion are compensated for by net acid absorption into the blood. This causes a localized acidosis in the intestinal venous blood, which can affect blood gas transport via the Bohr effect on haemoglobin affinity for oxygen. The absorbed H^+ ions also need to be subsequently excreted via the gills. Thus, there is an anatomical separation of net acid excretion (gills) and net base excretion (gut) in marine fish that is absent in freshwater fish. This phenomenon also causes an anatomical disconnect for the normally ~1:1 exchange of respiratory O_2 and CO_2 at the gills.

In seawater fish about 10% of their metabolic waste CO_2 is excreted via the gut as ionic HCO_3^- or solid $CaCO_3$. Living in hypersaline water further exaggerates this acid–base/respiratory disconnect between the gills and gut. The Arabian killifish (*Aphanius dispar*) is the teleost hypersalinity/high Ca^{2+} champion (175 ppt with 170 mM Ca^{2+}), taking gut carbonate excretion to a whole new level, and challenges the gills as the primary organ for handling respiratory CO_2.

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11:35 Friday 29th June 2012

A5.3
From fish carbonates to kidney stones: The role of multi-functional intestinal anion exchangers

Jonathan M. Whittamore (University of Florida, Florida, USA), Robert W. Freel (University of Florida, Florida, USA) and Marguerite Hatch (University of Florida, Florida, USA)

The cloning and characterization of the SLC26 family of anion exchangers has provided a valuable insight into the molecular underpinnings of Cl^- and HCO_3^- transport, serving a variety of essential functions in virtually all epithelia. The involvement of these transporters in the intestine has been recognized in marine fish, where they contribute to luminal alkalinization and subsequent production of calcium carbonate precipitates, with implications for osmoregulation and systemic calcium homeostasis.

Beyond Cl^-/HCO_3^- exchange, members of the SLC26 family can also transport a diverse range of anions. One of these is oxalate ($C_2O_4^{2-}$), the salt-forming anion of oxalic acid.

For vertebrates oxalate is non-functional, originating in part as an endogenous metabolic end-product, but a portion also comes from the diet. The burden of oxalate in the body is largely alleviated by the kidneys, however elevated levels in the urine are a major risk factor for calcium oxalate formation that comprises most (>75%) kidney stones. Although a problem for the kidney, the intestine is an established pathway for oxalate absorption and secretion, principally mediated by members of the SLC26 family of solute transporters. The transepithelial movement of oxalate by the intestine can also be modified by specialized oxalate-degrading bacteria present within the gut microflora. The intestine therefore makes a significant contribution to overall oxalate homeostasis.

Presently, regulation of the SLC26 anion exchangers is poorly understood as the mechanisms and signalling pathways involved have yet to be resolved. These are the focus of our investigations into oxalate transport by the intestine.

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12:00 Friday 29th June 2012

A5.4
The role of diet on marine fish calcium carbonate production

Erin E. Reardon (University of Exeter, UK), Nick Rogers (University of Exeter, UK), Christine Stephens (University of Exeter, UK), Louise

Lobb (University of Exeter, UK) and Rod W. Wilson (University of Exeter, UK)

Marine teleost fishes, which are not commonly known to be marine calcifiers, have been identified as an important source of calcium carbonate in the world's oceans. Unlike other marine calcifiers, marine teleosts excrete calcium carbonates as a by-product of drinking seawater to maintain their osmoregulatory balance. Current estimates suggest that fish contribute between 3 and 15% of global carbonate production, yet these estimates of calcium carbonate production rates are derived from samples collected from starved and resting individuals. Preliminary evidence suggests that carbonate production rates are positively correlated with drinking rate and metabolic rate. Thus, carbonate production rates are predicted to be higher in fed and/or active individuals – a more likely scenario for most fish in the wild. In addition, calcium carbonate chemistry and crystal morphology might vary with different diets. We tested these predictions across a range of temperate, marine teleost species by quantifying the relationship between calcium carbonate production rates, metabolic rate and diet. We also characterized calcium carbonate chemistry, morphology and solubility on a subset of samples produced. Ultimately, this work contributes to our goal of refining estimates of global fish carbonate production, describing their fate in the oceans, and how to integrate this information into our understanding of the global carbon cycle.

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12:25 Friday 29th June 2012

A5.5
Calcium absorption by the gastrointestinal tract of freshwater juvenile lake sturgeon (*Acipenser fulvescens*)

Janet Genz (University of Manitoba, Winnipeg, Canada), Benjamin Carriere (University of Manitoba, Winnipeg, Canada) and W. Gary Anderson (University of Manitoba, Winnipeg, Canada)

Calcium regulation in the cartilaginous freshwater lake sturgeon is limited by availability of both internal calcium stores and environmental calcium. In this study, we measured calcium fluxes in the gastrointestinal tract of juvenile lake sturgeon acclimated to 0.17, 0.34 and 2.26 mM environmental calcium, and compare the mechanisms of calcium transport in the anterior intestine and spiral valve. Net Ca^{2+} flux in the anterior intestine was 14.7 ±4.87, 12.3 ±3.49 and 13.5±1.94 pmol cm^{-2} h^{-1} at 0.17, 0.34 and 2.26 mM Ca^{2+}, respectively; while net transport in the spiral valve was 20.7 ±8.52, 16.5 ±4.99, and 21.4 ±8.92 pmol cm^{-2} h^{-1}. Blocking the apical epithelial calcium channel (ECaC) with ruthenium red (8.5 μM) significantly decreased Ca^{2+} influx in the anterior intestine at 0.17 and 2.26 mM Ca^{2+}, but exposure of the basolateral membrane to the Ca^{2+}-ATP-ase inhibitor trifluoperazine (10 mM) had no effect in any of the Ca^{2+} treatments. In contrast, ruthenium red and trifluoperazine appear to reduce Ca^{2+} uptake by the spiral valve in all treatment groups. The lack of effect of trifluoperazine on Ca^{2+} absorption in the anterior intestine, yet significantly higher Na^{+}/K^{+}-ATPase activity of anterior intestinal epithelia in fish exposed to 0.17 mM Ca^{2+}, suggests basolateral Ca^{2+} transport is dominated by Na^{+}–Ca^{2+} exchange over Ca^{2+}ATPase in lake sturgeon gastrointestinal tract.

Decreased Ca^{2+} absorption by both inhibitors in the spiral valve implies the mechanism of Ca^{2+} uptake differs along the length of the gastrointestinal tract, with regulation of Ca^{2+} balance possibly under greater control by distal, rather than proximal, intestinal segments.

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12:45 Friday 29th June 2012

A5.6
The multifunctional gut a haemodynamic perspective

Michael L. Axelsson (University of Gothenburg, Gothenburg, Sweden)

The evolution of the metazoans led to specialization of cells into discrete tissues and organs. Both the cardiovascular and gastrointestinal tract are examples of organ systems that represent a high level of organization that have enabled organisms to become more efficient and adapt to different environments.

The evolution of a gastrointestinal tract enabled meals of varying origin, composition and nutritional quality to be processed and more efficient digestion, absorption and internal distribution of nutrients. Animals in general show an enormous diversity in the morphology and physiology of the gastrointestinal tract, and fish – with over 30,000 species – are no different in this regard.

In parallel with the evolution of the gastrointestinal tract, cardiorespiratory support evolved and gastrointestinal blood flow is of critical importance to the effective digestion, absorption and distribution of nutrients. The blood supply to the gastrointestinal canal is closely regulated with respect to food intake and nutrient composition. Like any tissue, the gut requires an adequate blood flow that supplies oxygen and nutrients, along with the removal of carbon dioxide and other waste products. After feeding, there is an increased blood flow requirement in order to process the ingested food and support the activity of gastrointestinal uptake mechanisms.

Absorbed and metabolized/hydrolyzed nutrients must also be transported from the gastrointestinal mucosa to the liver, as well as to other parts of the intestine and beyond.

This presentation will summarize our current understanding of the close relationship between the cardiovascular and gastrointestinal system.

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14:00 Friday 29th June 2012

A5.7
Video analysis of gut motility: A useful tool to study environmental effects on food processing in fish?

Anna Holmberg (University of Gothenburg, Sweden) and Catharina Olsson (University of Gothenburg, Sweden)

The frequency and amplitude of gut motility are controlled by nerves and hormones, but are also affected by external factors such as food, pathogens, toxins and temperature. To better understand and predict the effects of environmental changes on food processing, we must study the integrated effects on gut motility.

So far, most studies in ectotherms have concentrated on smooth muscle contractions in an isolated region, and little is known about the existence of different motility patterns related to feeding status, for example. Video recording is commonly used to describe motility patterns in mammals and has previously also been applied to zebrafish larvae. Image analysis and construction of spatiotemporal maps can give the frequency, speed and direction of contractions. In this study, *in vitro* video recordings were used to characterize intestinal movements in adult zebrafish under different conditions. Propagation and local contractions were commonly found in the intestine. The activity was stable over several hours but could easily be manipulated with endogenous substances. Initially, nerve activity was blocked with tetrodotoxin, revealing a more uniform propagating pattern in the intestine suggestive of underlying slow-wave activity. Slow waves are pacemakers, setting the maximal contraction frequency in birds and mammals but have never been recorded in fish. Tetrodotoxin blocked most activity in the rectum, however, suggesting a different control mechanism.

In conclusion, video analysis of gut motility is useful for describing integrated contractile activity in fish as in mammals. Furthermore, nervous and non-nervous pathways control the gut motility patterns in fish.

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14:35 Friday 29th June 2012

A5.8
Effects of fasting and refeeding on the digestive physiology of selected sea bass populations

Jehan-Hervé Lignot (UMR5119, University of Montpellier 2, France), Maud Alix (UMR5119, University of Montpellier 2, France), Viviane Boulo (University of Montpellier 2, France) Bruno Guinand (UMR5554, Institute des Sciences de l'Evolution, France) and Béatrice Chatain (UMR110, CNRS, University of Nantes, France)

For teleost fish, the intestinal metabolic rate is at least five- to eight-fold higher than corresponding mass-specific whole-animal consumption rates. This is due to the multifunctionality of the gut and the energy-consuming roles of the enterocytes involved in digestive and osmoregulatory processes. While some individuals cope better with food deprivation, however, others recover more efficiently from prolonged fasting.

Using the strictly carnivorous European sea bass *Dicentrarchus labrax* L., we analysed the effects of feeding (2.5% of the individual body mass) on the postprandial osmoregulatory capacities and intestinal morphological changes using two groups of juveniles that demonstrated two inherited opposing trade-offs between tolerance to food deprivation and compensatory growth rate. Blood osmotic pressure, length of the intestinal folds, height of the enterocytes and brush border microvilli, lipid content, cell proliferation and localization of the sodium pump within the intestine were analysed for both groups in fasting condition (up to three weeks of food deprivation) and throughout the postprandial period. The results indicate a rapid post-feeding decrease in the osmoregulatory capacity of each phenotypic group and a differential time sequence for the postprandial digestive processes that also lead to distinct sensitivities to the fasting–refeeding conditions. Sea bass with better fasting tolerance therefore have reduced intestinal costs, but cannot optimize refeeding as well as individuals with more efficient compensatory growth rates that demonstrated larger up-regulation of their digestive capacities.

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15:00 Friday 29th June 2012

A5.9
Digestion under duress: Effects of hypoxia on nutrient absorption and metabolism in hagfish

Chris N. Glover (University of Canterbury, New Zealand), Carol Bucking (University of Ottawa, Ontario, Canada) and Chris M. Wood (McMaster University, Ontario, Canada)

Hagfish feed by immersing themselves in the body cavities of decaying animals. This ensures a rich nutrient source for uptake via the gut, and uniquely among vertebrates, the hagfish is also capable of absorbing organic nutrients via gills and skin. This feeding environment might also subject hagfish to reduced dissolved oxygen. The effect of hypoxia on nutrient assimilation and metabolism was investigated in the Pacific hagfish (*Eptatretus stoutii*) and the New Zealand hagfish (*E. cirrhatus*).

In response to hypoxia, plasma glucose levels increased in Pacific hagfish blood, but this effect was not mediated by an increase in gut glucose uptake, suggesting mobilization of tissue glycogen stores. In contrast, the absorption of glycine by the gut and gills was stimulated by hypoxia exposure. A 24-hour exposure to hypoxia in hagfish concurrently exposed to waterborne radiolabelled glycine led to a large increase (5.7-fold) in brain glycine accumulation, suggestive of a role for this amino acid in neural cytoprotection.

Feeding in New Zealand hagfish caused a three-fold elevation in metabolic rate. This specific dynamic action did not appear to be mediated by up-regulation in digestive enzymes and changes in intestinal structure. Hypoxia exposure following feeding eliminated this metabolic increase.

These data indicate that hagfish can selectively enhance the assimilation of nutrients that might assist with critical functions during hypoxia, but that the bulk of digestion might be delayed until a return to normoxic waters.

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15:20 Friday 29th June 2012

A5.10
Stomach loss in the Tetraodontiformes

Jonathan M Wilson (CIIMAR, Portugal), Odete M. Goncalves (CIIMAR, Portugal) and L. Filipe C. Castro (CIIMAR, Portugal)

The stomach, characterized by acid-peptic digestion, is a highly successful vertebrate innovation. The absence of gastric glands has been identified in a number of teleost fish lineages, however, indicating that secondary loss has occurred multiple times.

In the Tetraodontiformes, the digestive function of the pufferfish (crown sister families Tetradontidae and Diodontidae) stomach has been lost, and instead the stomach is used for their characteristic inflation behaviour, which is used to defend against predation. The absence of acid-peptic digestion and gastric glands correlates with gastric gene loss (*Atp4a* and *Atp4b* genes). The inflation behaviour of pufferfishes is an example of a major functional innovation whose origin might lay in the evolutionary transition from coughing, to water blowing, to inflation in the Tetraodontiformes. It is unclear, however, whether stomach inflation arose before or after loss of the gastric glands, since the absence of gastric glands in basal families remains unclear.

To clarify the pattern of stomach loss in the Tetraodontiformes, we examined specimens from the more basal families: Balistidae (*Rhinecanthus aculeatus*), Monacanthidae (*Paraluteres prionurus*) and Ostraciidae (*Lactoria cornuta*) in addition to the crown family Tetraodontidae (*Tetraodon nigroviridis*) for the presence of *Atp4a* by real-time polymerase chain reaction using degenerate primers and gastric gland by histology and immunohistochemistry. We confirm the absence of the gastric phenotype in Tetraodontidae as well as in basal Ostraciidae; however, gastric glands and Atp4a are present in the foregut of the intermediate sister families Balistidae and Monacanthidae.

These results indicate that stomach loss occurred at least twice in the Tetraodontiformes.

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16:10 Friday 29th June 2012

A5.11
Diuretic and antidiuretic strategies in the blood-gorging bug, *Rhodnius prolixus*

Ian Orchard (University of Toronto, Ontario, Canada) and Angela Lange (University of Toronto, Ontario, Canada)

Serotonin is a diuretic hormone in *R. prolixus*, but there is also ample evidence for the presence of a peptidergic diuretic hormone. The identity of this peptide has remained elusive; but recently, using molecular techniques and mass spectrometry, we have sequenced the peptidergic diuretic hormone and found it to be a member of the CRF-related family of insect neuropeptides. Rhopr-CRF/DH has potent biological activity on the anterior midgut and Malpighian tubules, and works synergistically with serotonin during rapid post-feeding diuresis in *R. prolixus*.

We have also identified a peptide, Rhopr-CAPA-2, and its receptor that are important mediators in the cessation of rapid post-feeding diuresis in *R. prolixus*, inhibiting serotonin-stimulated secretion by Malpighian tubules, but not, apparently, Rhopr-CRF/DH-stimulated secretion. The antidiuretic hormone Rhopr-CAPA-2 appears to prevent the synergism between the diuretic hormones serotonin and Rhopr-CRF/DH.

The interplay between diuretic and antidiuretic hormones on the digestive system in *R. prolixus* results in a remarkable diuresis; a diuresis that has evolved to eliminate excess water and salts from a massive blood meal, while maintaining homeostasis within the haemolymph.

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16:35 Friday 29th June 2012

A5.12
Intestinal perfusion demonstrates high paracellular nutrient absorption in an insectivorous bat, *Tadarida brasiliensis*

Edwin R. Price (University of Wisconsin-Madison, Wisconsin, USA), Antonio Brun (Universidad Nacional de San Luis, Argentina), Verónica Fasulo (Universidad Nacional de San Luis, Argentina), William H. Karasov (University of Wisconsin-Madison, Wisconsin, USA) and Enrique Caviedes-Vidal (Universidad Nacional de San Luis, Argentina)

Water-soluble nutrients can be absorbed transcellularly across enterocyte membranes via protein-mediated transport, or paracellularly through the tight junctions between enterocytes. Previous *in vivo* measurements of bats that were orally dosed with passively-absorbed carbohydrate probes have shown that bats absorb larger proportions of nutrients paracellularly than similarly-sized non-flying mammals. While this could indicate greater paracellular permeability of the intestinal epithelium, it could also be caused by longer retention time or slow gastric evacuation.

To determine whether bat intestines are particularly permeable to nutrient-sized molecules, we performed *in situ* intestinal luminal perfusions on *Tadarida brasiliensis*. We cannulated the intestine and recirculated an isosmotic buffer containing 10–75 mM D-glucose, 10–75 mM proline, and two carbohydrate probes that are only absorbed paracellularly, 1 mM L-arabinose, and 1 mM lactulose, and radioisotope tracers for these molecules.

Absorption of arabinose (MW 150) was nearly double that of lactulose (MW 342), demonstrating a similar molecular size-sieving effect, as has been seen previously for various species *in vivo*. At low molarity proline conditions, paracellular absorption (assessed by arabinose clearance) can account for at least 44% of total proline absorption. At 75 mM proline, paracellular absorption accounts for at least 66% of proline absorption.

These data demonstrate that *Tadarida brasiliensis* relies heavily on paracellular absorption for the uptake of nutrients and confirms the high intestinal permeability suggested by whole-animal studies.

Supported by NSF Award 1025886.

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17:00 Friday 29th June 2012

A5.13
Diverse gut microbes facilitate ingestion of dietary toxins in herbivores

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For decades, it has been hypothesized that gut microbes play a role in the detoxification of plant secondary compounds (PSCs) consumed by herbivorous hosts. To test this, we investigated whether evolutionary history of the host with PSCs shapes microbial diversity and whether gut microbes facilitate ingestion of PSCs. We investigated the microbial communities of two woodrats (*Neotoma bryanti* and *N. lepida*).

For each species, we compared experienced populations that independently converged to feed on the same toxic plant (creosote, *Larrea tridentata*) to naïve populations with no prior exposure to creosote toxins. Naïve and experienced populations of both species were fed either a control diet or a diet containing 2% extracted creosote resin. Foregut contents were collected for microbial 16S rRNA sequencing. A subset of *N. lepida* was given an antibiotic treatment, and food intake and body mass were monitored.

Ingestion of creosote resin increased the diversity (Shannon index) as well as abundance of *Bacteroidetes* in experienced hosts of both species but not in naïve hosts. Woodrats given antibiotics consumed less food and lost more weight compared to woodrats not given antibiotics, but only when the diet contained creosote resin.

These results indicate that responses of the gut microbiota to PSCs depend on experience of the host with PSCs, and that responses are convergent across species. We hypothesize that adaptation of the microbiota to creosote PSCs in experienced woodrats drives these differential responses. Additionally, the antibiotic study represents the first experimental evidence that microbes enhance the consumption of PSCs in wild herbivores.

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17:20 Friday 29th June 2012

A5.14
Ontogeny and dietary effect on gene expression of intestinal carbohydrases in birds with the altricial mode of development

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In birds, developmental changes in digestive enzymes occur during transition from a lipid-rich yolk diet inside the egg to a carbohydrate- and protein-based diet post hatching. Carbohydrase activity, which is critical for starch digestion, increases per unit intestine under control of intrinsic regulatory ('hard-wired') mechanisms and, in some species, environmental signals such as increased dietary starch (called dietary induction). We predicted that in altricial birds such increases are paralleled by increases in expression of genes, which is the case in mammals and chickens.

House sparrows and zebra finch nestlings were each raised on two diets that differed in starch content. Seemingly hard-wired increases in intestinal maltase and sucrase activity prior to fledging occurred in the absence of significant increases in mRNA for enzymes maltase-glucoamylase and sucrase-isomaltase, in contrast to our prediction. Carbohydrase activities were induced by dietary starch only in the house sparrow, and increased activity was correlated with increased mRNA for both maltase-glucoamylase and sucrase-isomaltase, suggesting transcriptional control of the diet-induced response.

Post-fledging changes were then studied in house sparrows. Parallel up and down changes in carbohydrase activities and mRNA were inducible by dietary carbohydrate changes in house sparrow fledglings, demonstrating reversible flexibility under apparent transcriptional control.

We conclude that in altricial birds ontogenetic changes in enzyme activity are under the control of both transcriptional and post-transcriptional mechanisms.

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Poster Session – Saturday 30th June 2012

A5.15
Identification and physiological characterization of Nesfatin-1 in the rat heart

Tommaso Angelone (University of Calabria [UoC], Italy), Tommaso Angelone (UoC, Italy), Filice Elisabetta (UoC, Italy), Teresa Pasqua (UoC, Italy), Amodio Nicola (University of Catanzaro, Italy), Michele Galluccio (UoC, Italy), Gabriella Montesanti (UoC, Italy), Anna M. Quintieri (UoC, Italy), Maria C. Cerra (UoC, Italy)

Nesfatin-1, a peptidic fragment derived from nucleobindin 2 (NUCB2), is known for its anorexic effects. *NUCB2* gene expression is regulated by nutritional status, indicating a regulatory role of peripheral Nesfatin-1 in energy homeostasis. Nesfatin-1 is able to cross the blood–brain barrier and this suggests that peripheral sources can affect brain activity or that central Nesfastin-1 can regulate peripheral functions. Its influence on alimentary behaviour, its peripheral distribution and the endocrine release, however, lead us to hypothesize a role in the processes that accompany obesity.

So far nothing is known concerning the role of Nesfatin-1 at cardiac level. By using Western blotting techniques, we identified the presence of Nesfatin-1 in rat cardiac extracts. Physiological analyses performed on isolated and Langendorff-perfused paced cardiac preparations revealed that exposure to Nesfatin-1 induces negative inotropic and lusitropic effects, without affecting coronaries. These effects involve particulate guanylate cyclase (pGC), protein kinase G (PKG) and are independent due to nitric oxide synthase activation. Nesfatin-1 increased ERK1/2 phosphorylation, with no effects on protein kinase B, eNOS, and nNOS phosphorylations, and protein S-nitrosylation.

In conclusion, we suggested that:

- the heart expresses Nesfatin-1;
- Nesfatin-1 directly affects myocardial performance; and
- cardiac effects are mediated by the pGC/PKG pathway and ERK1/2.

Results might pave the way to include Nesfatin-1 in the neuroendocrine modulators of cardiac function and encourage clarification of its clinical potential in the presence of nutrition-dependent physiopathological conditions, including obesity.

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Poster Session – Saturday 30th June 2012

A6 – ECOLOGICAL ASPECTS OF FATTY ACIDS

A6.1
Membrane fatty acids: The influence of dietary fats and the relation between membrane composition, basal metabolism and maximum longevity

A.J. Hulbert (University of Wollongong, Australia)

Fatty acids are essential for life not because they are an energy source but because they are essential components of membranes. In contrast to saturated and monounsaturated fats, both omega-6 and omega-3 polyunsaturated fats (PUFAs) are independently essential in the diet of higher animals.

Recent studies in rats suggest that in normal situations membrane fatty acid composition is to a large extent homeostatically regulated irrespective of diet fatty acid composition. It appears that it is the balance between omega-6 and omega-3 PUFAs that has the greatest influence on membrane composition. Unlike membrane lipids, diet has a strong influence on the fatty acid composition of storage lipids (triglycerides). There are patterns between species in membrane fatty acid composition that are proposed to relate to species differences in basal metabolism and maximum lifespan. It is suggested that the influence of membrane composition on cell metabolic activity is due to the physical properties that fatty acids give to membrane bilayers. In contrast, it is suggested that it is the differential chemical susceptibilities of fatty acids to lipid peroxidation that is responsible for the relation between membrane composition and maximum longevity.

Examples of both interspecific and intraspecific correlations between membrane composition and maximum longevity of vertebrates and invertebrates will be presented. Examples where there is a link between other aspects of membrane lipid composition (plasmalogens and non-methylene-interrupted fatty acids) and exceptional longevity will also be briefly discussed.

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10:30 Saturday 30th June 2012

A6.2
Changes in tissue fatty acid composition during fasting in vertebrates

Edwin R. Price (University of Wisconsin-Madison, Wisconsin, USA)

While fat content consistently decreases during fasting in vertebrates, changes in fatty acid composition during fasting are poorly studied. I will view potential changes in fatty acid composition through the lens of two processes that can be predicted to be important during fasting: selective mobilization of fatty acids from adipocytes, and changes in the triacylglycerol-to-phospholipid ratio. A literature review reveals that these two processes can explain many of the observed changes in the fatty acid composition of fasting animals. Thus, they can provide a baseline of expected changes by which we can judge exceptional species.

I report data from one such exceptional species: the 13-lined ground squirrel (*Ictidomys tridecemlineatus*). Hibernating species often selectively retain polyunsaturated fatty acids, a pattern that contrasts the more typical retention of saturated fatty acids and mobilization of unsaturated fatty acids. Isolated adipocytes from 13-lined ground squirrels reflect the pattern seen *in vivo* in hibernators, retaining unsaturated fatty acids and mobilizing saturates. These exceptional species might indicate unique regulatory mechanisms for fatty acid mobilization within adipocytes.

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11:20 Saturday 30th June 2012

A6.3
Cold-hearted creatures: Polyunsaturated fatty acids affect cardiac function in hibernators

Carla Frare (University of Veterinary Medicine [UVM], Austria), Sylvain Giroud (UVM, Austria), Arjen M. Strijkstra (University of Groningen, Netherlands), Ate S. Boerema (University of Groningen, Netherlands), Walter Arnold (UVM, Austria) and Thomas Ruf (UVM, Austria)

Polyunsaturated fatty acids (PUFAs) have strong effects on hibernation and daily torpor. Increased dietary uptake of PUFAs of the omega-6 class lengthens the duration of torpor and enables animals to reach lower body temperatures and metabolic rates. We hypothesized that this well-known influence of PUFAs might be mediated through the effects of the membrane fatty acid composition on sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (SERCA IIa) in the heart of hibernators. Specifically, based on published data from non-hibernators, we expected that increased proportions of omega-6 fatty acids in the surrounding membrane should increase the activity of this trans-membrane Ca^{2+} pump, allowing continued function of the heart at body temperatures as low as 1°C.

We determined SERCA activity (at 37ºC) in hearts collected from hibernating Syrian hamsters (*Mesocricetus auratus*), along with natural inter-individual variation in SR phospholipid fatty acid composition. We found that SERCA activity significantly (p<0.001) increased as the ratio of omega-6 to omega-3 PUFAs increased. In particular, SERCA activity was positively affected by high proportions of linoleic acid (C18:2 omega-6; p<0.001) and was negatively affected by docosahexaenoic acid (C22:6 omega-3; p<0.001). Interestingly, the omega-6 to -3 PUFA ratio in SR was significantly higher (p<0.001) in hibernating than in normothermic hamsters, suggesting an active remodelling of myocyte SR membranes as the animals entered torpor.

We conclude that the PUFA composition of membranes affects cardiac function, and hence the minimum body temperature tolerated by hibernators. We suggest that the need to recreate degraded SERCA IIa protein might explain periodic arousals during hibernation.

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11:55 Saturday 30th June 2012

A6.4
Effects of dietary fatty acids on metamorphosis in winter flounder (*Pseudopleuronectes americanus*)

Marie Vagner (UMR, LIttoral ENvironnement et Sociétés, France), Benjamin De Montgolfier (CEHTRA, France), Jean-Marie Sévigny (Maurice Lamontagne Institute, Quebec, Canada), Réjean Trenblay

(UQAR, Institut des Sciences de la Mer de Rimouski, Quebec, Canada) and Céline Audet (UQAR, Institut des Sciences de la Mer de Rimouski, Quebec, Canada)

The aim of this study was to investigate the effects of dietary highly unsaturated fatty acid (HUFA) content on the fatty acid composition of juvenile winter flounder (*Pseudopleuronectes americanus*) and the ensuing consequences on the expression of genes involved in key metabolic processes developing during metamorphosis.

Three groups of fish were fed from the pelagic larval stage until 30 days after settlement on rotifers enriched with different blends of microalgae providing different HUFA profiles: the Nanno diet was rich in eicosapentaenoic acid (EPA) and arachidonic acid (AA), the Tiso diet was rich in docosahexaenoic acid (DHA), and the cocktail diet was rich in EPA, DHA and AA.

Fish growth performance was not limited by the dietary HUFA contents. Growth hormone gene expression seemed to depend almost entirely on the DHA dietary content at settlement, however, while EPA and AA were required in addition to DHA after settlement to sustain it. Fifteen days after settlement, oxidative defences measured through *superoxide dismutase* (*sod*) gene expression decreased in the cocktail group, while it remained constant in the Tiso and Nanno groups at the same time. This would indicate that different EPA, AA and DHA dietary content would have an impact on the reactive oxygen species production in fish cells.

Taken together, these results allow a better understanding of the effect of dietary HUFA on the juvenile metamorphosis process of winter flounder, which is a promising candidate for coldwater marine aquaculture in North America.

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12:30 Saturday 30th June 2012

A6.5
Do polyunsaturated fatty acids impact on lactation in MF1 mice?

Julia Diels (Veterinary University, Vienna), Nadine Lenzhofer (Veterinary University, Vienna) and Teresa Valencak (Veterinary University, Vienna)

Lactation is not only the most energy-demanding period for female mammals, but is also known to induce hyperthermia in the animals as all organs are working at sustained levels and they have to synthesize milk on top of this. Thus, laboratory mice from the MF1 strain are limited by their capacity to dissipate heat while raising young. Identifying ways in which heat dissipation can improved will likely improve the lactation performance of females.

Polyunsaturated fatty acids (PUFAs) are essential dietary constituents that mammals cannot synthesise *de novo*. Once ingested, however, they can be further elongated and desaturated enzymatically. PUFAs play an important role as constituents of biological membranes, as signalling molecules, and they influence torpor and hibernation in mammals. Based on these findings, we hypothesized that PUFAs might relate to body temperature. To test the idea, we subcutaneously implanted transponders into 45 female MF1 mice that also allow for skin temperature readings. After three weeks, we provided the animals with three isocaloric diets that were enriched with saturated, omega-3 or omega-6 PUFAs. After a couple of weeks and daily measurements of skin temperature, we paired a subset of the females with males and later observed them throughout lactation by assessing skin temperature, food intake, litter mass and body weight.

Our results might help in understanding the regulation of body temperature during lactation.

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12:50 Saturday 30th June 2012

A6.6
Metabolome in obesity and its comorbidities

Matej Oresic (VTT Technical Research Centre of Finland, Finland)

Primary obesity and psychotic disorders and their treatments share similarities in their associated changes in energy balance and cardiometabolic comorbidities, including metabolic syndrome. The specific underlying mechanisms linking the expansion of adipose tissue to these comorbidities, however, are unknown. These disease associations do not demonstrate causal links, but instead suggest that specific causes of and metabolic disturbances associated with obesity could also play a pathogenic role in these comorbidities, potentially even before obesity develops. Both brain and peripheral metabolic organs use metabolites including lipids as components of their integrated homeostatic system to control energy balance as well as in regulating their peripheral insulin sensitivity. Metabolomics has played an important role in elucidating the underlying pathways behind obesity and its comorbidities, including diabetes and psychotic disorders as well as cancer and Alzheimer's disease. Knowledge of common and specific mechanisms could help in aetiopathogenic understanding, early disease detection and the identification of individuals who might benefit from specific treatments to prevent or treat the comorbidities of obesity. Such knowledge might also lead to the discovery of unexpected novel therapeutic avenues.

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13:50 Saturday 30th June 2012

A6.7
On the allometric scaling of fatty acids in the phospholipids of metabolically-active fowl tissues

András Szabó (Kaposvár University, Hungary), Miklós Mézes (Szt. István University, Hungary), Krisztián Balogh (Szt. István University, Hungary), Róbert Romvári (Kaposvár University, Hungary) and Hedvig Fébel (Research Institute of Animal Breeding and Nutrition, Hungary)

In our recent studies, domesticated fowl species in the range from 150 g (Japanese quail, *Coturnix coturnix japonica*) to 19 kg (turkey, *Meleagris gallopavo*) were analysed to elucidate the supposed allometric relationship of the membrane lipid fatty acids (FAs). The basis of all studies was the theory of 'membranes as pacemakers of metabolism' by Hulbert (*Lipids*, 2007; 42: 811–819). Negative allometric scaling was found for docosahexaenoic acid (DHA) first in the myocardium (B = -0.6), later in the avian kidney (-0.18) and lung (-0.24), and in the liver (-0.2). In the membrane FAs of all these tissues, balanced polyunsaturation, negatively scaling n3 and unsaturation index and negatively related n6 and monounsaturated FA molar levels were described.

In the lavaged avian lung surfactant phospholipids, we found similar negative allometry for DHA. In contrast, avian brain phospholipid FA composition failed to provide body mass relation. We found unexpected results (positive allometry for n3 FAs, DHA and unsaturation index) in the m. pectoralis superficialis phospholipids during turkey ontogenesis. In the aforementioned splanchnic organs the concentration of whole-tissue malondialdehyde was negatively related to body mass (B=-0.16, -0.05, -0.17 and -0.13 in the heart, lung, kidney and liver, respectively).

Results indicate a special regulatory role for DHA, in agreement with the membrane pacemaker theory, while suggest a strong predisposition and linking role for this acid and the polyunsaturated n3 FAs towards non-enzymatic lipid peroxidation.

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14:40 Saturday 30th June 2012

A6.8
Role of highly unsaturated fatty acids on hypoxia tolerance of the golden grey mullet (*Liza aurata*)

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In the natural environment, omega-3 highly unsaturated fatty acids (n-3 HUFAs) are only weakly synthesized *de novo* in marine fish and therefore must be supplied by food. The main natural source of n-3 HUFAs is microalgae. Microalgae production largely depends on chemical and physical conditions and is therefore subject to change as part of global change. The n-3 HUFAs, and in particular eicosapentaenoic and docosahexaenoic acids, are the main component of cell membranes in fish. In consequence, their availability the diet is linked to their levels in membranes, and consequently in the physiological performance of fish.

In this context, the aim of this study was to evaluate the effect of n-3 HUFA dietary content on the physiological performance, in terms of tolerance to hypoxia, of golden grey mullet (*Liza aurata*), a microalgae grazer. Two replicated groups of fish were fed on a n-3 HUFA-rich diet (1.0% EPA plus DHA on a dry matter basis) or a n-3 HUFA-poor diet (0.1% EPA plus DHA on a dry matter basis) for four months at 20°C.

Hypoxia tolerance was tested on 10 fish per replicate (i.e. 20 per experimental condition) by progressively decreasing air saturation from normoxia (>85% air saturation) to severe hypoxia using nitrogen. At each air saturation level tested, the number of fish loosing equilibrium and the number of fish performing aquatic surface respiration was recorded and those fish were removed from the tank.

The results will be discussed in the context of evaluating the adaptive capacity of *Liza aurata* in a variable environment.

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Poster Session – Saturday 30th June 2012

A6.9
Fatty acid content and composition are different in clinically-healthy dogs and in dogs with otitis externa

Magdalena Siemieniuk (University of Bialystok, Poland), Urszula Czyzewska (University of Bialystok, Poland), Adam Tylicki (University of Bialystok, Poland), Pawel Dobrzyn (Nencki Institute of Experimental Biology, Poland) and Agnieszka Dobrzyn (Nencki Institute of Experimental Biology, Poland)

Malassezia pachydermatis is isolated from clinically-healthy dogs as well as from the cases of otitis externa. The pathogenicity of *M. pachydermatis* is mainly associated with metabolic, hormonal or immunological disorders in the host as a consequence of changes in lipids on the skin surface. To date, it is not fully established whether the expression of the virulence is determined only by environmental factors or pathogenic forms have other, independent mechanisms essential for the virulence.

The aim of the present study was to examine the fatty acid (FA) content and composition in samples of *M. pachydermatis* isolated from dogs with (n=30) or without (n=31) otitis externa. *M. pachydermatis* was identified using Sabouraud media with chloramphenicol, morphological features and polymerase chain reaction-restriction fragment length polymorphism. FA profiles were analysed by gas–liquid chromatography.

The total FA content was increased by 35% in strains isolated from clinically-healthy dogs compared to strains obtained from sick dogs. Additionally, fungi isolated from healthy dogs had significantly higher intracellular saturated FA content. This increase was mainly caused by elevation in the levels of arachidonic, palmitic, palmitoleic and stearic acids.

The results obtained show differences in FA content and composition between *M. pachydermatis* isolated from healthy and sick dogs. FA analysis might therefore be helpful tool that will allow us to identify potentially pathogenic strains for monitoring and implementation of preventive procedures before the occurrence of clinical signs of disease.

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Poster Session – Saturday 30th June 2012

A6.10
Body temperature in the Ames dwarf mouse: Is it affected by dietary fatty acids?

Nadine Lenzhofer (University of Veterinary Medicine, Austria), Julia Diels (University of Veterinary Medicine, Austria) and Teresa Valencak (University of Veterinary Medicine, Austria)

The Ames dwarf mouse is a long-lived mutant mouse that – in comparison to its heterozygous siblings – lives up to five years. Mutation at the Prop1 locus (*Prop1df*) results in a reduced body size, a lack of thyroid stimulating hormone, growth hormone and prolactin in the animals. Homozygous Ames dwarf mice have also been reported to have a 1.6°C lower core body temperature than heterozygous controls. Phenotypically, the Ames dwarf resembles mice under caloric restriction.

Polyunsaturated fatty acids (PUFAs) are essential dietary components and membrane constituents that have a huge impact on cellular functions. Among PUFAs, it is particularly important to distinguish between omega-3 and -6 PUFAs, as these two classes are said to affect metabolism in different ways. Recently, our group observed that membrane fatty acid composition in the Ames dwarf mouse has different omega-3 and omega-6 PUFA content, with the dwarfs having membranes rich in omega-6, comparatively poor levels of omega-3 polyunsaturated fatty acid. We thus hypothesized that the differences in membrane fatty acid composition would correspond to the lower body temperature in the Ames dwarf mice.

We implanted temperature-sensitive transponders subcutaneously into all of our experimental Ames dwarf mice and started to feed the animals three isocaloric, but different, diets: a saturated one, another rich in omega-6 PUFAs and a third one rich in long-chain omega-3 PUFAs. We measured temperature, body weight and behaviour in all mice to determine whether membrane fatty acid composition affects body temperature in Ames dwarf mice.

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Poster Session – Saturday 30th June 2012

A7 – TRANSEPITHELIAL SOLUTE TRANSPORT

A7.1
Ammonia excretion, sodium uptake, cortisol and intracellular signalling during high environmental ammonia exposure in fish

Chris M. Wood (McMaster University, Ontario, Canada), C. Michele Nawata (McMaster University, Ontario, Canada), Amit K. Sinha (University of Antwerp, Belgium), H. Jung Liew (University of Antwerp, Belgium) and Gudrun De Boeck (University of Antwerp, Belgium)

Researchers have been arguing over the mechanisms of ammonia transport across the gills of fish for more than 70 years, ever since the original work by August Krogh. With recent discoveries that Rh glycoproteins are expressed in fish gills, that they bind NH_4^+ but transport NH_3, and that the H^+ ions move separately by mechanisms coupled to active Na^+ uptake, thereby facilitating NH_3 diffusion, it now appears that there is a functional Na^+/NH_4^+ exchange complex – i.e. Krogh's original ideas were close to the truth.

The mechanism is activated by ammonia loading, such that ammonia can be excreted against apparent gradients. Recent *in vivo* experiments with trout, carp and goldfish have shown that Na^+ uptake and efflux are both up-regulated during chronic high environmental ammonia exposure, along with various mRNAs of the Na^+/NH_4^+ exchange complex. Cortisol mobilization appears to play a key role. Using *in vitro* models, we are studying the signalling involved in this activation in pavement cells of freshwater trout, where increased gene expression of apical Rhcg2 appears to be particularly important. Stimulation by elevated ammonia alone is sufficient to increase the level of Rhcg2 transcripts in the pavement cells while cortisol and the mineralocorticoid 11-deoxycorticosterone have potentiating effects on this transcriptional response. The effects appear to involve cAMP mobilization, but neither eplerenone nor RU-486 block the response, suggesting a non-genomic action.

Current focus is on possible transcriptional regulation of Rhcg2 by cAMP response element binding via the ERK (MAP kinase) signalling pathway.

(NSERC Discovery, CFI, CRC Program, IWS-BOF University of Antwerp, Belgium).

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10:30 Sunday 1st July 2012

A7.2
Mechanisms of copper-induced inhibition of ammonia excretion and sodium uptake in fish

Alex M. Zimmer (McMaster University, Ontario, Canada), Indianara F. Barcarolli (Federal University of Rio Grande, Brazil), Chris M. Wood (McMaster University, Ontario, Canada) and Adalto Bianchini (Federal University of Rio Grande, Brazil)

Copper is a potent toxicant to aquatic organisms, eliciting physiologically toxic effects at sub-lethal levels. The most common of these responses are a disruption in ammonia excretion (J_{amm}) and sodium uptake ($J_{Na\ in}$). In the current model for ammonia excretion, the enzymes Na^+/K^+-ATPase, H^+-ATPase and carbonic anhydrase potentially link these processes mechanistically.

In a recent study, for the first time in vivo we demonstrated a significant inhibition of carbonic anhydrase by copper after freshwater- and seawater-acclimated guppies native to Southern Brazil were exposed to copper (20 µg/l) for 12 hours. This inhibition occurred concomitantly with a reduction in J_{amm} at both salinities. Following 96 hours of exposure, both carbonic anhydrase activity and J_{amm} were fully recovered.

We hypothesized that copper-induced inhibition of carbonic anhydrase is a phenomenon that occurs early during copper exposure and is recovered relatively quickly due to the physiological importance of this enzyme. In rainbow trout, however, carbonic anhydrase activity was unaffected by copper over 24 hours of exposure despite significant inhibition of J_{amm} for the majority of exposure and a significant inhibition of $J_{Na\ in}$ from three to nine hours of exposure. Interestingly, in both species, H^+-ATPase activity increased significantly over the duration of the exposure, potentially implicating this enzyme in the restoration J_{amm} and/or JNa in during sub-lethal copper exposure.

(NSERC Discovery Grant, IDRC, CNPq, INCT-TA, NSERC CRD, IZA, ILZRO, NiPERA, ICA, CDA, Teck Resources, and Vale Inco.)

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11:00 Sunday 1st July 2012

A7.3
A novel pattern of smoltification in the most anadromous salmonid: Pink salmon (*Oncorhynchus gorbuscha*)

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Pink salmon migrate immediately to seawater (SW) following gravel emergence at a very small size (0.2 g – the smallest of all anadromous salmonids). It is not known whether they pre-adapt to SW entry through a process of smoltification. Previous studies demonstrated that SW entry was associated with a two- to three-fold elevation in whole-body ion levels, indicating that they night not smolt.

In this study, post-hatch pink salmon were held in FW throughout development or transferred to SW every two weeks for 20 weeks. SW tolerance progressively increased as indicated by percentage survival and maintenance of whole-body water content and $[Na^+]$ following SW transfer. FW held fish exhibited increases in gill Na^+/K^+-ATPase (NKA) activity and α-1b mRNA NKA isoform expression levels and a reduction in that for the α-1a isoform over this time period.

Survival following direct transfer to SW was 100% at yolk-sac absorption (12 weeks post-hatch) and then decreased shortly thereafter, defining a window of salinity tolerance. Smoltification was not complete at yolk-sac absorption, however, as SW transfer was associated with a further eight-fold increase in gill NKA α-1b/α-1a expression. Thus, the ontogeny of salinity tolerance is a two-stage process centred on yolk-sac absorption and requiring SW entry to complete the smoltification process. In fish held continuously in FW throughout development, there was a dramatic increase in whole-body ion levels around the time of yolk-sac absorption, indicating a fundamentally different pattern of smoltification than that of other anadromous salmonids.

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11:20 Sunday 1st July 2012

A7.4

Inositol is an essential metabolite for osmoregulation in seawater-adapted euryhaline teleosts

Gordon Cramb (University of St Andrews, Scotland, UK), Svetlana Kalujnaia (University of St Andrews, Scotland, UK), Steven Gellatly (University of St Andrews, Scotland, UK) and Neil Hazon (University of St Andrews, Scotland, UK)

Myo-inositol is a major osmolyte, which plays an important role in cyto-protection in many organisms exposed to a variety of environmental stresses, including exposure to elevated salinity. Cells exposed to hypertonic environments can either synthesize myo-inositol from glucose 6-phosphate via the actions of the two enzymes myo-inositol phosphate (MIP) synthase and myo-inositol monophosphatase (IMPA) or accumulate the cyclic alcohol from extracellular fluids by the actions of sodium- and/or proton- mediated myo-inositol transporters (SMIT and HMIT respectively). Studies have identified species- and tissue-specific increases in the expression in certain MIP synthase, IMPA, SMIT and HMIT isoforms when euryhaline fish move from freshwater to seawater. In general, the IMPA1 isoform is markedly up-regulated in epithelial tissues exposed to the marine environment, and enhanced expression is also detected in various internal organs such as the kidney and gonad. In eels and tilapia, IMPA1 expression was located throughout the basal/germinal cell layers within the stratified epithelia of the oesophagus, skin, fin and branchial arch and primary filaments, the interstitial cells and/or glomerulae between renal tubules. Specifically in eels, it is also located in branchial and fin ray chondrocytes. In most tissues examined, the increases in expression of MIP synthase, IMPA1 and SMIT and HMIT after seawater-transfer were correlated with elevated tissue inositol contents. These results suggest that increases in intracellular inositol concentrations are essential for cell volume regulation in various endothelial, epithelial and epidermal tissues when fish are exposed to the dehydrating effects of the seawater environment.

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11:50 Sunday 1st July 2012

A7.5

Ca^{2+} – a versatile messenger in an insect model for transepithelial transport: The salivary glands of the blowfly

Kristoffer Heindorff (University of Potsdam, Germany), Bernd Walz (University of Potsdam, Germany) and Otto Baumann (University of Potsdam, Germany)

Fluid secretion in salivary glands of the blowfly, *Calliphora vicina*, is induced by the neurohormone serotonin (5-HT). Binding of 5-HT to two distinct G-protein-coupled receptors leads to coactivation of the InsP3/Ca^{2+} and the cAMP signalling pathways (Berridge and Heslop, *Br. J. Pharmacol.* 1981; 73: 729–738), thereby promoting the secretion of a KCl-rich saliva. We focus on the signalling mechanisms that mediate the 5-HT-dependent secretory response.

Intracellular Ca^{2+} increases the permeability of the epithelium for Cl^- (Prince and Berridge, *J. Exp. Biol.* 1972; 56: 323–333). Recent studies, however, indicate multiple and more complex modulatory functions of Ca^{2+} in intracellular signalling. We demonstrate that Ca^{2+} affects cAMP signalling via a Ca^{2+}/CaM-dependent adenylyl cyclase. Together with a previously reported positive effect of cAMP on Ca^{2+} signalling (Schmidt *et al.*, *BMC Physiol.* 2008; 8: 10), this crosstalk between signalling pathways might function as an amplifier to optimize transepithelial transport. Moreover, our results indicate that Ca^{2+} forms a negative feedback loop via the protein phosphatase calcineurin, which seems to desensitize Ca^{2+} signalling for 5-HT.

In conclusion, we demonstrate a novel degree of complexity regarding Ca^{2+}-dependent processes involved in fine-tuning of intracellular signalling in the salivary glands of the blowfly.

Funded by Deutsche Forschungsgemeinschaft (Wa 463/9-6).

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12:10 Sunday 1st July 2012

A7.7

Cell volume responses of ionocytes of eurythermal killifish are blocked by cold shock but not after cold acclimation: Fragility of complex regulatory pathways

William S Marshall (St Francis Xavier University, Nova Scotia, Canada), Hannah Buhariwalla (St Francis Xavier University, Nova Scotia, Canada), Katelyn Barnes (St Francis Xavier University, Nova Scotia, Canada) and Regina Cozzi (St Francis Xavier University, Nova Scotia, Canada)

Chloride cells in the opercular epithelia (OE) of the euryhaline, euryoxic, and eurythermic killifish (*Fundulus heteroclitus*) actively secrete chloride, an energetic process that can be easily measured electrophysiologically as short-circuit current (Isc). NaCl secretion involves the Na,K,2Cl cotransporter (NKCC) in the basolateral membrane in series with anion channels (CFTR) in the apical membrane and both transporters are activated by focal adhesion kinase-1 phosphorylated at tyrosine 407 (FAK pY407) in turn activated by integrin-based osmosensor.

Hypotonic shock rapidly and reversibly inhibits NaCl secretion. Chilling OE to 8ºC or lower blocks the osmosensitive response, but if fish are acclimated to 5ºC for more than four weeks, fatty acid composition changes and OE from cold-acclimated fish remain osmosensitive in the cold. NaCl secretion continues in cold-exposed OE, albeit at lower rates: Arrhenius plots demonstrate a sharp non-linearity below 8ºC in aerobic conditions, but less in anaerobic, cyanide-treated OE, implying a mitochondrial inner membrane source of nonlinear behaviour.

The FAK-mediated regulation of OE ion transport is apparently blocked by a phase change in plasma membrane lipid, but in cold-acclimated OE the maintenance of liquid phase membranes keeps the normal regulation of ion transport operating in the cold.

Supported by NSERC.

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12:30 Sunday 1st July 2012

A7.8

The multifunctional role of Rhesus proteins in nitrogen excretion, sodium uptake and CO_2 transfer in zebrafish

Steve F. Perry (University of Ottawa, Ontario, Canada)

Fish that inhabit freshwater continually lose salts across permeable body surfaces that are replenished by active absorption across specialized ion-transporting cells of the gills of adults or the skin of larvae. This talk will focus predominantly on the cellular and molecular mechanisms of sodium uptake in zebrafish larvae and its linkage to ammonia excretion.

The cells responsible for Na^+ uptake in zebrafish larvae are a sub-type of mitochondrion-rich cell that are enriched with V-type H^+-ATPase; these cells are termed HR cells. Two molecular models have been proposed to account for Na^+ uptake; one involving epithelial Na^+ channels energetically linked to H^+-ATPase and another incorporating electroneutral Na^+/H^+ exchange (NHE).

Despite apparently unfavourable chemical gradients, the results of

recent research favour the NHE model. It is suggested that a functional metabolon linking NHE with ammonia excretion via an apical membrane ammonia channel, Rhcg1, facilitates Na^+ uptake via the specific NHE isoform, NHE3b. Recent research suggests that the Rhcg1 protein might also play a significant role in CO_2 excretion. Rates of Na^+ uptake and ammonia excretion are tightly regulated according to environmental conditions by a suite of neurochemicals and hormones including cortisol and catecholamines.

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13:50 Sunday 1st July 2012

A7.9

The effects of low pH water on ion regulation in Brown-striped marsh frogs', *Limnodynastes peronii*, larvae

Rebecca L. Cramp (University of Queensland, Australia), Edward A. Meyer (University of Queensland, Australia) and Craig E. Franklin (University of Queensland, Australia)

For fish and aquatic amphibian larvae, survival in acidic waters presents a significant challenge with waters of pH 5 and less inhibiting active sodium uptake, damaging epithelia and increasing diffusive efflux of electrolytes across the gills and integument. Recent studies have identified molecular ion transporters in the gills and integument of amphibian larvae that might be affected by exposure to low pH water and therefore contribute to the disruptions in electrolyte transport and loss of membrane function in acid-exposed larvae.

The present study examines the impact of low pH exposure on ion regulation and on the expression several key epithelial ion transporters in the brown-striped marsh frog, *Limnodynastes peronii*. In *L. peronii* larvae reared in neutral artificial soft water, acute exposure to pH 3.5 artificial soft water results in a marked suppression of sodium uptake rates and a marked increase in sodium efflux rates; after 24 hours of acute exposure to low pH water, approximately 60% of larvae had perished. Acute exposure of larvae reared in neutral artificial soft water to a sub-lethal pH (pH 4.5) resulted in no mortality. The mRNA of genes for key branchial and integument ion transporters (Na^+/K^+-ATPase, epithelial sodium channel and vacuolar H^+-ATPase) have recently been identified in the gills/integument of *L. peronii* larvae; the effects of prolonged exposure to sub-lethal low aquatic pH on the gene expression of these ion transporters will also be detailed.

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14:20 Sunday 1st July 2012

A7.10

How did ion regulation arise? Using hagfish to define mineral regulation in vertebrates

Greg Goss (University of Alberta, Alberta, Canada), Alex Clifford (University of Alberta, Alberta, Canada), Aaron Schultz (University of Alberta, Alberta, Canada), Brendan Goss (University of Alberta, Alberta, Canada), James Ede (University of Alberta, Alberta, Canada) and Nic Bury (Kings College London, UK)

Considered to be an extant representative of early life history, the hagfish (*Eptatretus stoutii*) is part of the monophyletic linage of craniates that diverged from the vertebrates ~600million years ago. Thus, hagfish present an interesting model for the study of evolution in early vertebrates.

Unlike higher vertebrates, hagfish only possess a single corticosteroid receptor. Research shows that the hagfish corticosteroid receptor is activated by a number of different mineralo- and glucocorticoids in *in vitro* assays. The ancestral corticosteroid ligand and the corticosteroid receptor function, however, have not been elucidated.

To investigate the evolution of ion regulation, we use hagfish as a model species, since they are known to regulate divalent ions but not monovalent ions. We hypothesize that divalent ion regulation is the early mineralocorticoid response that is later co-opted for monovalent ion regulation. To determine the active steroid, we injected hagfish with varying amounts of cortisol, corticosterone, 11-doxycorticosterone and 11-deoxycortisol and monitored plasma glucose, ATPase activity and plasma ions (Na^+, Cl^- and Mg^{2+}). Furthermore, the ability of the animals to recover plasma SO_4^- following injection of SO_4^- ions as a mineral stress was determined by monitoring for a stress response of elevated glucose and elevated ion secretion rates.

Our results demonstrate that hagfish can raise a stress response and increase active sulphate secretion rates. While this appears to be a steroid-controlled active glucocorticoid and mineralocorticoid response, however, its identity remains to be elucidated.

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14:40 Sunday 1st July 2012

A7.11

Solute driving forces for water transport in the marine teleost intestine

Jonathan M. Whittamore (University of Florida, Florida, USA) and Rod W. Wilson (University of Exeter, UK)

To overcome dehydration, marine teleost fish drink seawater. The intestine therefore has an established role in osmoregulation, effectively absorbing fluid from ingested seawater. While intestinal fluid transport has been almost exclusively characterized as driven by Na^+-linked solute absorption, for teleosts substantial net HCO_3^- secretion also contributes directly through apical Cl^-/HCO_3^- exchange. In addition, the alkaline conditions created by HCO_3^- secretion within the gut precipitate Ca^{2+} as insoluble $CaCO_3$, helping maintain the osmotic gradient for water absorption.

While theoretical and experimental data support this prediction, more recent studies suggest there are additional mechanisms. The majority of secreted HCO_3^- is produced endogenously from intracellular CO_2 with preferential basolateral H^+ extrusion. Analysis of data from *in vivo* intestinal perfusions with varying [Ca^{2+}] from 1 mM (low) to 90 mM (high), suggest that when $CaCO_3$ precipitation is limited there is a shift to apical H^+ secretion (titrating luminal HCO_3^-), sustaining water absorption.

In paired *in vitro* gut sac experiments (with no interference from $CaCO_3$ precipitation) mucosal application of bafilomycin (to inhibit V-H^+-ATPase) resulted in a significantly drop in fluid absorption of over 40%. Furthermore, as seawater is processed along the intestine, levels of Mg^{2+} and SO_4^{2-} increase, reaching up to 150 mM *in vivo*. We hypothesized that the unusually low osmotic coefficient of $MgSO_4$ permits this accumulation while still supporting fluid absorption. Additional gut sac experiments where increasing [$MgSO_4$] was replaced with mannitol revealed that $MgSO_4$ is osmotically less expensive to accumulate, but may also have more than a passive influence on fluid absorption

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15:00 Sunday 1st July 2012

A7.12

Immunohistochemical approach to understanding branchial ion regulation in Magadi tilapia (pH10)

Jonathan M Wilson (Ecofisiologia CIMAR, Porto, Portugal), Chris M. Wood (McMaster University, Ontario, Canada), Pierre Laurent (McMaster University, Ontario, Canada), Claudine Chevalier (McMaster University, Ontario, Canada), Harold L. Bergman (University of Wyoming, Wyoming, USA), Adalto Bianchini (Universidade Federal

do Rio Grande, Brazil), John N. Maina (University of Johannesburg, South Africa), Ora E. Johannsson (Fisheries and Oceans Canada, Ontario, Canada), Lucas F. Bianchini (Universidade Federal do Rio Grande, Brazil), Geraldine D. Kavembe (South Eastern University College, Kenya), Michael B. Papah (University of Nairobi, Kenya) and Rodi O. Ojoo (University of Nairobi, Kenya)

The small cichlid *Alcolapia grahami* of Lake Magadi, Kenya, thrives under extremely inhospitable aquatic conditions (pH ~10, alkalinity ~300 mequiv/L, up to 42ºC and diel hypo- to hyperoxic conditions). In the Fish Spring lagoon, where A. grahami are most abundant, water [Na^+], [Cl^-] and osmolality are 356 mM, 112 mM and 525 mOsm/kg, respectively. *A. grahami* show typical marine fish osmoregulatory characteristics, although the dominant anion is HCO_3^- rather than Cl^-. The gills are designed for a high O_2 uptake capacity and mitochondrion-rich cell (MRC) subtypes have been identified in the filament epithelium from electron microscope studies. In the present study we have used immunohistochemistry to characterized MRC subtypes based on staining for ion transport proteins using heterologous antibodies to CFTR anion channel, Na^+:K^+:2Cl^- cotransport (NKCC)/Na^+:Cl^- cotransporter (NCC), Na^+:HCO_3^- cotransporter (NBC) and Na^+/K^+-ATPase (NKA). The typical chloride cell is present with an apical CFTR anion channel and basolateral NKCC1 and NKA (= type4 MRC). NCC apical staining is present in cells with weak NKA and strong NBCe1 basolateral staining (type2 MRC) and a third MRC type with only strong NKA staining (type 1) was also identified. We were unable to identify a tilapine type3 MRC (apical NHE3 and basolateral NKA), which has been associated with acid excretion and sodium uptake. In contrast to other tilapines, *A. grahami* is atypical in having coexpression of type4 and type2 MRCs, which are typically associated with Cl^- excretion and uptake, respectively. Instead, we propose type4 MRCs are involved in HCO_3^- excretion and type2 MRCs in Cl^- uptake.

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15:40 Sunday 1st July 2012

A7.13
The ClC-3 chloride channel and hyperosmoregulation in the European sea bass, *Dicentrarchus labrax*

Maryline Bossus (Université de Montpellier 2, France), Guy Charmantier (Université de Montpellier 2, France) and Catherine Lorin-Nebel (Université de Montpellier 2, France)

The European sea bass, *Dicentrarchus labrax*, is exposed to salinity changes due to its migrations between the sea and estuaries/lagoons. In euryhaline teleosts, the mechanisms of hyperosmoregulation are still debated, particularly the channel responsible for Cl^- uptake. The chloride channel 3 (ClC-3), which is highly conserved in vertebrates, has been proposed as a potential fish osmosensor due to its activation by hypotonicity and to its role in regulatory volume decrease in various cell types. Its potential involvement in hyper-osmoregulation and osmosensing is investigated in this study. Branchial and renal ClC-3 mRNA expressions were significantly lower in fresh water than in sea water shortly after transfer from fresh water to sea water from 10 minutes on and for up to 30 days. At the protein level, however, branchial ClC-3 increased significantly after a hypotonic shock, seven days after transfer to fresh water. This increase is partially due to an increased number of mitochondria-rich cells and also to the increased ClC-3 amount within each cell. ClC-3 colocalized with the basolateral Na^+/K^+-ATPase in the mitochondria-rich cells and in the renal collecting ducts and tubules. The ClC-3 was immunodetected in gills at all conditions. In the kidney however, ClC-3 immunolocalization was only detected after seven days in fresh water. The differential expression of this chloride channel according to salinity within the osmoregulatory tissues suggests its involvement in osmoregulation through Cl^- uptake and cell volume regulation.

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16:00 Sunday 1st July 2012

A7.14
Effect of metabolic rate on EE2 uptake in *Fundulus heteroclitus*

Tamzin A Blewett (McMaster University, Ontario, Canada), Lisa Robertson (McMaster University, Ontario, Canada), Deborah MacLatchy (Wilfrid Laurier University, Ontario, Canada) and Chris Wood (McMaster University, Ontario, Canada)

The aim of this study was to determine whether metabolic rate influences 17a-ethynylestradiol (EE2) uptake in male *Fundulus heteroclitus*, potentially via a similar mechanism to oxygen uptake through the lipophilic gills. EE2 – the major component of birth control – cannot be fully broken down by sewage treatment, resulting in varying background water levels. F. heteroclitus were exposed to100 ng/L radiolabeled [^{3}H]EE2 for two hours while swimming at 0, 15 and 40 cm/s, and in a subsequent experiments killifish were exposed to both hypoxic and hyperoxic conditions (P_{O2} <80 mmHg, and P_{O2} ≥ 500 mmHg) while oxygen consumption (M_{O2}) and EE2 accumulation were measured.

A positive correlation between M_{O2} and EE2 accumulation was seen (R2=0.997, p.002) and more EE2 was taken up during the 40 cm/s swim than at the lower swim speeds, meaning that oxygen uptake is a predictor of EE2 uptake in *F. heteroclitus* under normoxic conditions. EE2 tended to accumulate in the liver (where toxicants are metabolized in the gall bladder and enter bile), and the gut (where bile is received). In the hypoxia exposures, the pattern of uptake remained the same in the tissues, however, there was a large increase in uptake compared to normoxia-exposed fish, and M_{O2} decreased while EE2 uptake increased. Hyperoxia resulted in no change in EE2 uptake from normoxia conditions.

These data will be useful in developing a predictive model to determine optimal timing and location of wastewater treatment plant discharges.

(NSERC Strategic Grant, D. MacLatchy, P.I.)

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16:20 Sunday 1st July 2012

A7.15
Transport of selenium across the plasma membranes of hepatocytes and enterocytes of rainbow trout (*Oncorhynchus mykiss*)

Sougat Misra (University of Saskatchewan, Saskatchewan, Canada), Raymond W.M. Kwong (University of Saskatchewan, Saskatchewan, Canada) and Som Niyogi (University of Saskatchewan, Saskatchewan, Canada)

Transport of essential solutes across biological membranes is one of the fundamental characteristics of living cells. Although selenium is an essential micronutrient, little is known about the cellular mechanisms of chemical species-specific selenium transport in fish. Here we report the kinetic and pharmacological transport characteristics of selenite and its thiol (glutathione and L-cysteine) derivatives in the primary hepatocytes and enterocytes of rainbow trout.

Findings from the current study suggest an apparent low-affinity linear transport for selenite in both cell types. In the presence of glutathione, however, we recorded a saturable high-affinity Hill kinetics (K_d = 3.61 ±0.28 μmol^{-1}) in enterocytes. The uptake of selenium in the presence of thiols was several-fold higher than the uptake of selenite alone (at equimolar concentration) in both cell types. Transport of selenite and its thiol derivatives were energy independent. We observed a decrease in selenite transport with increasing pH and *vice versa* in the presence glutathione in these cell types. A competitive inhibition of selenite uptake was recorded in the presence of a structurally similar compound, sulphite. The uptake of selenite as well as its thiol derivatives was found to be sensitive to the anion transport blocker, irrespective of the cell types. Inorganic mercury (Hg^{2+}) elicited an inhibition of selenite transport in

these cells, but augmented the transport of reduced forms of selenium in hepatocytes.

Based on the substrate choice and comparable pharmacological properties, we advocate that multiple anion transport systems are probably involved in the cellular transport of selenite in fish.

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16:40 Sunday 1st July 2012

A7.16
Whole-body calcium regulation of larval freshwater lake sturgeon (*Acipenser fulvescens*) during growth

Janet Genz (University of Manitoba, Winnipeg, Canada), Cheryl Klassen (University of Manitoba, Winnipeg, Canada) and W. Gary Anderson (University of Manitoba, Winnipeg, Canada)

In low calcium environments, bony fish can rely on skeletal sources for the maintenance of appropriate calcium levels. The cartilaginous, freshwater lake sturgeon, however, is limited by both internal sources of calcium and low calcium availability in the external environment.

The high calcium demand during the early life stages makes this a critical period for calcium uptake. This study considers the effect of external calcium concentrations on the developmental growth of larval lake sturgeon. Larvae were raised from fertilization in three environments that differed in available [Ca^{2+}] (0.17, 0.34 and 1.78 mM), with 0.34 mM representing the natural habitat of the adults. Larval whole-body calcium influx and efflux, mass and total length were measured at each developmental stage until the transition to exogenous feeding. Larval sturgeon had similar growth for the first 11 days post-hatch, but at 25 and 35 days post-hatch larvae exposed to 1.78 mM Ca^{2+} weighed significantly less than the fish raised in 0.17 mM Ca^{2+}. Total length of larvae did not vary due to environmental [Ca^{2+}], however, implying the difference in growth might be due to slower reabsorption of the yolk in the hypocalcaemic group. Larval sturgeon in the 0.17 mM environment demonstrated Ca^{2+} influx rates over 20-fold greater than larvae exposed to natural environmental [Ca^{2+}] (0.34 mM), and at least 110-fold greater than fish raised in 1.78 mM Ca^{2+} at all post-hatch time points, indicating that even at this low environmental concentration larval lake sturgeon had sufficient adaptive scope to survive and grow.

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17:00 Sunday 1st July 2012

A7.17
Drilling for nutrition: The physiological mechanism of bone penetration by *Osedax*

Sigrid Katz (Scripps Institution of Oceanography, UC San Diego [UCSD], California, USA), Martin Tresguerres (Scripps Institution of Oceanography, UCSD, California, USA) and Greg W. Rouse (Scripps Institution of Oceanography, UCSD, California, USA)

Annelids belonging to Siboglinidae lack a gut and obtain nutrition via bacterial symbionts housed in a specialized organ called the trophosome. While most siboglinids host chemoautotrophic symbionts, which allow them to thrive in reducing habitats such as hydrothermal vents or methane seeps, *Osedax* exploits vertebrate bones lying on the seafloor. In contrast to other siboglinids, *Osedax* house heterotrophic Oceanospirillales bacteria in their posterior body, which are modified into so-called 'roots'. These roots penetrate and ramify through the bone, which serves as their food source (Goffredi *et al*., 2007).

Osedax lack any obviously bioabrasive structures and the physiological mechanism of bone erosion and nutrient absorption was virtually unknown. The ultrastructure of the root epidermis suggests secretory/absorptive functions of this region and we hypothesized that *Osedax* demineralize the bone by secreting acid, followed by absorption of bone collagen and lipids for nutrition. Our analysis of putative acid-secreting proteins, namely vacuolar H^+-ATPase (VHA) and carbonic anhydrase (CA), by immunohistochemistry and quantitative immunoblotting, shows preferential location and high abundance of VHA in the root epidermal cells. CA also co-occurs with VHA in the root epidermis, and is found in other cells and body regions, suggesting that CA is also involved in maintaining the acid–base balance throughout the worm.

These results support our hypothesis on bone erosion via acid secretion by *Osedax*, which is similar to chemical mechanisms employed for boring by some gastropods and for bone demineralization by human osteoclasts.

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Poster Session – Sunday 1st July 2012

A7.18
Zebrafish (*Danio rerio*) osmo/ionoregulatory mechanism is modulated by cortisol acting on skin ionocyte differentiation and proliferation

Shelly Abad Cruz (Academia Sinica Taipei, Taiwan, R.O.C.), Pei Lin Chao (Academia Sinica Taipei, Taiwan, R.O.C) and Pung-Pung Hwang (Academia Sinica Taipei, Taiwan, R.O.C)

In teleost fish, skin/gill function during environmental acclimation is well-known to be regulated by cortisol as an adaptive mechanism. Since glucocorticoid (GC) and mineralocorticoid (MC) were linked to osmo/ionoregulation and teleost fish do not posses aldosterone, we examined the molecular pathway using zebrafish – one of the premier molecular models.

This study aims to set a new platform clarifying the involvement of cortisol possibly playing the role of GC and MC along with their respective receptors during epidermal cells development affecting osmo/ionoregulation. Treatment with exogenous cortisol (20 mg/L) for 24–72 hours from zero hours post fertilization (hpf) in zebrafish embryos caused in increase in mRNA spatial expressions of forkhead transcription factors 3 (foxi3a and foxi3b), the epidermal ionocyte progenitor development regulators. As a consequence, there were significantly increased epidermal ionocyte densities of Na^+/K^+-ATPase rich cells and H^+-ATPase-rich cells, along with up-regulated mRNA levels of ionocyte gene markers. Only the glucocorticoid receptor mRNA was down-regulated upon cortisol incubation. No changes were found in the mineralocorticoid receptor. In gene knockdown, only loss of glucocorticoid receptor caused a significant decrease in the number of epidermal stem cells, Na^+/K^+-ATPase rich cells, H^+-ATPase-rich cells, keratinocytes and mucus cells. No changes were found in mineralocorticoid receptor morphants.

In conclusion, cortisol via glucocorticoid receptor alone transactivates the foxi3a/b that favours the ionocyte progenitor specification–differentiation process, causing the increased matured ionocyte numbers. These findings are evidence of the stimulated function of cortisol acting as glucocorticoid and mineralocorticoid in osmo/ionoregulation.

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Poster Session – Sunday 1st July 2012

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A8 – A NEW VISION OF CEREBROSPINAL FLUID IN THE DEVELOPMENT AND FUNCTION OF THE CENTRAL NERVOUS SYSTEM

A8.1
Cerebrospinal fluid flow through the ventricles and subarachnoid space is critical to normal development of the cerebral cortex

Jaleel A. Miyan (University of Manchester, UK)

Different lines of evidence are presented from human and animal, *in vivo* and *in vitro* observations indicating that proper production, flow and drainage of cerebrospinal fluid (CSF) is required for normal development of the cerebral cortex. CSF volume output initiates at the start of cortical development and is not required for brain stem and spinal cord development, which is served by embryonic CSF (aka neural tube fluid). CSF is a growth medium for neural progenitor cells driving proliferation and differentiation with a composition that changes with embryonic age. CSF flow into the subarachnoid spaces outside the brain is also shown to be critical to normal development, in particular to migration of neurones into the cortical plate.

A number of conditions of deficient or abnormal development can be explained as a consequence of abnormalities in the CSF system. A unique folate handling system has been identified that supplies the cerebral cortex alone and utilizes the CSF. This system appears to be exquisitely sensitive to CSF drainage issues, which can effectively lead to a folate deficiency. Thus, an understanding of the role of CSF in cortical development might produce preventative strategies for many different neurological conditions.

This talk summarises 15 years of research by many research associates in the lab, without whom we would not be where we are now.

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11:00 Friday 29th June 2012

A8.2
The power of pressure: Driver of embryonic brain growth?

Mary E. Desmond (Villanova University, Pennsylvania, USA)

The vertebrate embryonic brain develops as a hollow structure similar to a balloon. Accumulation of cerebrospinal fluid via an osmotic gradient creates internal pressure and supplies trophic factors to the neuroepithelium. The neuroepithelium grows as an active, interdependent synergism between the increase of cerebrospinal fluid within the cavity and proliferation of the primitive neurons comprising the neuroepithelium.

A decrease in the intra-luminal pressure of the chick embryonic brain results in 50% reduction in both the tissue volume and cell number. An increase in intra-luminal pressure elicits a two-fold increase in the cell number. How does the tension across the neuroepithelium created by the pressure of the brain stimulate the nucleus to divide? Might there be mechanoreceptors on the basal or luminal surface of the embryonic neuroepithelium, such as focal adhesion kinases (FAKs) that respond to changes in tension via integrin signalling. These non-receptor kinases are known to respond to an increase in fluid stress across the apical surface of blood vessels facing fluid. FAKs have been found on the endothelium lining blood vessels and on lung alveolar epithelium.

Desmond and Gato have preliminary data using the chick embryo suggesting that the pattern of phosphorylated FAK (tyr 397) across the neuroepithelium differs when the internal pressure is increased.

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11:50 Friday 29th June 2012

A8.3
Water and ion transporters in the choroid plexus: Insight from knockout mice

Jeppe Praetorius (Aarhus University, Denmark) and Helle H. Damkier (Aarhus University, Denmark)

The choroid plexus epithelium secretes the major proportion of the intraventricular cerebrospinal fluid. Most of the secretion occurs through the transcellular movement of water and solutes from the blood side, although paracellular transport might modulate net transport.

A number of integral plasma membrane proteins have been mapped to the choroid plexus epithelium, which are specialized for water and ion transport. The Na^+/K^+-ATPase is situated in the luminal membrane and directly pumps Na^+ into the ventricles. Other Na^+ and K^+ transporters include NKCC1, Ncbe, Nhe1, NBCe2, KCCs and K^+ channels. Cl^- and bicarbonate are transported by Ncbe, AE2, NBCe2 and Cl^- channels. Water seems to mainly pass though the AQP1 channel.

Our presentation will provide an overview of studies that have been conducted over the past decade to verify the involvement of many of these proteins in cerebrospinal fluid secretion by the choroid plexus.

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12:25 Friday 29th June 2012

A8.4
Anatomical, molecular and functional characterization of the embryonic blood–cerebrospinal fluid barrier at early stages of brain development

Maryam Parvas (Shahid Beheshti University, Iran) and David Bueno (Barcelona University, Catalonia, Spain),

In vertebrates, early brain development takes place at the expanded anterior end of the neural tube. After closure of the anterior neuropore, the brain wall forms a physiologically-sealed cavity that encloses embryonic cerebrospinal fluid (E-CSF), a complex and protein-rich fluid that is initially composed of trapped amniotic fluid. Most of the proteins contained within the E-CSF, which play crucial role in central nervous system development, are transferred form the blood serum (Parvas *et al*., 2008a). E-CSF has several crucial roles in brain anlagen development.

We have reported the presence of a transient blood–CSF barrier located

in the brain stem lateral to the ventral midline, at the mesencephalon and prosencephalon level in chick and rat embryos by transporting proteins, water, ions and glucose in a selective manner via transcellular routes (Parvas *et al*., 2008b; Parvas and Bueno, 2010a; Parvas and Bueno, 2010b). We have blocked the activity of this barrier by treating the embryos with 6-aminonicotinamide gliotoxin (6-AN). We demonstrate that 6-AN treatment in chick embryos blocks protein and water transport across the embryonic blood–CSF barrier and influences neuroepithelial cell behaviour, as monitored by neuroepithelial progenitor cell survival, proliferation and neurogenesis (Parvas and Bueno, 2011).

Our results contribute to delineate the actual extent of this blood–CSF embryonic barrier controlling E-CSF composition and homeostasis, and the actual importance of this control for early brain development. They also elucidate the mechanism by which proteins and water are transported thought transcellular routes across the neuroectoderm, reinforcing the crucial role of E-CSF for brain development.

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13:00 Friday 29th June 2012

A8.5
Instructive cues for neural stem cells in the cerebrospinal fluid

Maria K. Lehtinen (Children's Hospital and Harvard Medical School, Massachusetts, USA), Mauro Zappaterra (Harvard Medical School, Massachusetts, USA), Ping Ye (University of North Carolina at Chapel Hill, North Carolina, USA), Joseph D'Ercole (University of North Carolina at Chapel Hill, North Carolina, USA), Eric T. Wong (Beth Israel Deaconess Medical Center, Massachusetts, USA), Anthony LaMantia (The George Washington University, Washington, USA) and Christopher A. Walsh (Children's Hospital and Harvard Medical School, Massachusetts, USA)

Neural stem cells located along cerebrospinal fluid (CSF)-filled ventricles serve as building blocks for the mammalian brain. The importance of genes in guiding neural stem cell division is clear: mutations in single genes affecting cell division can profoundly impact brain size, leading to microcephaly (small brain). Genes are not enough, however, as all cells integrate their genetic programs of cell division with environmental cues.

Given the CSF's immediate proximity to neural stem cells, we have explored how the CSF provides a dynamic set of environmental signals to instruct neurogenesis. We found that the CSF imparts age-dependent effects on neurogenesis. Embryonic CSF triggers robust stem cell division, while CSF obtained at other ages, including adult CSF, stimulates only limited cell division.

As expected, many signals identified in the CSF are age-dependent. For example, we found that CSF-insulin-like growth factor-2 (IGF2) expression peaks during brain development. At this time, CSF-IGF2 binds to its receptors on the surface of neural stem cells instructing them to divide.

Our findings are clinically relevant as well. For example, CSF-IGF2 concentrations from glioblastoma multiforme patients were correlated with disease progression. They suggest a model where the embryonic CSF coordinates with neural stem cells to regulate neurogenesis. Since the CSF is home to hundreds of proteins, the instructive roles of CSF are likely to expand beyond IGF activity and neural stem cell division.

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14:00 Friday 29th June 2012

A8.6
Embryonic CSF: Effects on neuroepithelial progenitor cell behaviour during early brain development

Angel Gato (Universidad de Valladolid, Spain) and David Bueno (University of Barcelona, Catalonia, Spain)

Embryonic cerebrospinal fluid (E-CSF) exhibits a crucial and specific role during early brain development. In this communication, we will present general and particular data on the control exerted by E-CSF on neuroepithelial progenitor cell patterning. We demonstrate that E-CSF has a direct effect on neuroepithelial progenitor cell behaviour, promoting cell survival, proliferation and neurogenesis, as well as on particular gene expression.

The search for factors contained within E-CSF has revealled the presence of FGF-2, which has an effect on the regulation of neuroepithelial cell behaviour, mainly on cell proliferation and to some extent on neurogenesis. This molecule could have an extraneural origin, being selectively transported to the brain cavity, thus suggesting a new and complementary way of regulating brain development.

Using proteomics analysis, we identified 26 and 30 different gene products in the E-CSF from chick and rat embryos, respectively, most of which were involved in the regulation of brain developmental processes during embryogenesis in systems other than E-CSF. One of these proteins was retinol binding protein – an all-*trans* retinol carrier – that we demonstrate to be present within the E-CSF. We show that in chick embryo brain development, E-CSF has retinoic acid (RA) activity, which suggests that this fluid may act as a diffusion pathway for RA. It also helps to control RA synthesis in the mesencephalic–metencephalic isthmus, thus inducing embryonic brain neurogenesis.

Taken together, these and other results that will be discussed demonstrate that E-CSF shows specific properties that could be useful for neural progenitor cell handling.

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14:50 Friday 29th June 2012

A8.7
The role of folate metabolism in foetal onset hydrocephalus

Besma Nash (University of Manchester, UK), Irum Naureen (University of Manchester, UK) and Jaleel Miyan (University of Manchester, UK)

Delivery and availability of folate to the developing cerebral cortex is critical for normal development. Foetal onset hydrocephalus (FOHC) is characterized by an imbalance in the absorption and secretion of cerebrospinal fluid (CSF) *in utero*, leading to abnormal cerebral cortex development resulting in severe neurological deficits. The aim of our study is to identify whether a folate imbalance is also present in human FOHC.

Infant samples were collected via either lumbar puncture or ventricular tap, spun down immediately and frozen at -80°C. Western blotting/dot blots were used to determine the amounts of folate enzymes and products in the CSF. Samples were analysed for total protein and total folate.

Our data indicate a significant decrease in FDH in FOHC CSF. These data corroborate rat data, where a similar decrease was found in the hydrocephalic rat (Cains *et al*., 2009). There is a significant decrease in homocysteine and cysteine in the methionine cycle. There is a general decrease, although not significant, in other methionine cycle intermediates. Total folate analysis using the microbiological assay indicates a trend for lower total folate concentrations in FOHC CSF.

These data are the first to show the presence of folate/methionine metabolites in foetal human CSF, suggesting the existence of a unique handling system to supply the developing brain. The folate and methionine cycles are crucial for the survival and maturation of cells. These observations may explain the poor cortical development and neurological deficits in infants with FOHC. Our future aims are to address this reduction in pregnant women with supplementation.

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15:25 Friday 29th June 2012

A8.8

Cerebrospinal fluid formation measured by ventriculocisternal perfusion method: A fact or artefact?

Marijan Klarica (University of Zagreb, Croatia)

Cerebrospinal fluid (CSF) formation, unidirectional circulation and absorption represent the classic hypothesis of CSF hydrodynamics. It is generally believed that CSF formation is an active process that occurs mainly in choroid plexuses. CSF formation rate (V_f) has been extensively studied using the ventriculocisternal perfusion technique. This method and the equation for the calculation of V_f were established by Heisey *et al.*, who assumed that the dilution of the indicator substance is a consequence of newly-formed CSF, i.e. that a higher CSF formation rate will result in a higher degree of dilution of the indicator substance. This method is therefore indirect and any dilution of the indicator substance in the perfusate caused by other reasons would result in questionable and often contradictory conclusions regarding CSF formation rates.

In spite of the general acceptance of this hypothesis, there is a considerable series of experimental results that do not support the idea of the active nature of CSF formation and suggests that choroid plexuses inside the brain ventricles are the main places of formation. In our laboratory, a new ventriculo-aqueductal perfusion method has been introduced for the determination of CSF formation inside the isolated brain ventricles. On one hand, it provides the possibility of direct insight into CSF formation, and on the other, it clearly indicates that there is no net CSF formation inside the brain ventricles.

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16:15 Friday 29th June 2012

A8.9

Role of folate in normal and abnormal brain development: CSF drainage as a target for folate therapy in congenital hydrocephalus

Alicia Requena Jimenez (University of Manchester, UK)

Cerebrospinal fluid (CSF) flow in the brain is maintained thanks to a balance between CSF production from the choroid plexus and CSF absorption by the arachnoid granulations draining CSF into the venous sinus. Abnormal folate provision due to impairment of this mechanism in congenital hydrocephalus has been directly associated with changes in folate enzyme profiles in CSF. We have shown that the resulting folate deficiency can be alleviated through maternal folate supplementation; importantly, this cannot be achieved with folic acid.

The principal aim of the current study is to investigate this unique folate delivery system and to understand its role in the central nervous system. Understanding how the system operates might allow the design of better supplements for a worldwide neurological condition affecting 1/5,000–1/280 newborn babies.

A sequential study of the regional distribution of 10-formyl tetrahydrofolate dehydrogenase (FDH), the main folate-binding protein in the CSF along with folate transporters within the brain, was investigated using immunohistochemistry. In relation to treatment and its association with drainage improvement, a study of the effect of hydrocephalic CSF on normal arachnoid cells was carried out during embryonic days.

We hypothesize that folate transport by FDH could present an alternate mechanism to choroid plexus transporters. Furthermore, the FDH–folate transport might be altered in hydrocephalus due to a low FDH provision by CFS or decreased in situ biosynthesis. We also hypothesize that our treatment will allow increased drainage at the level of the arachnoid membrane through the proliferation of active drainage cells, thereby re-establishing CSF circulation.

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Poster Session – Saturday 30th June 2012

A9 – PHYSIOLOGY OF ENVIRONMENTAL GRADIENTS

A9.1

Climbing downhill backwards: understanding how animals we don't fully know respond to environments we can't fully measure

Julian D. Metcalfe (Centre for Environment Fisheries Aquaculture Science, UK) and David A. Righton (Centre for Environment Fisheries Aquaculture Science, UK)

The biological capabilities of animals are usually measured under controlled laboratory conditions that focus on the responses of individuals, usually in a steady-state and frequently to only one or two stressors. But does this fully reflect how that animal, in a wild population, will respond where its state (feeding, migrating, breeding, etc.) might change and where it is continuously exposed to a myriad of varying, complex and potentially conflicting stressors? Telemetry, using electronic devices attached to, or implanted into, the animal offers the capability to record its movements and behaviour while simultaneously recording at least some of the environmental variables to which it is exposed. We shall consider how recent advances in telemetry enable these measurements to be recorded from marine fish over seasonal and even multi-annual timescales. This new information can then be integrated with our understanding of fish physiology to improve our capacity to predict how individuals and, ultimately, populations might respond to environmental gradients.

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10:30 Sunday 1st July 2012

A9.2

A thermodynamic framework for modelling energy budgets across environmental gradients

Michael R. Kearney (University of Melbourne, Australia)

An important challenge in the study of climatic limits and adaptation lies in connecting measurements of climatic tolerance to life history consequences in the context of natural microclimatic gradients. An increasingly rich array of spatiotemporal data on global environmental data has developed over the past 30 years. It is difficult to connect such data directly to empirical measurements of climatic tolerance, especially in terrestrial environments, because organsimal temperature and water balance are nonlinear functions of air temperature and humidity.

In this talk I will show how thermodynamically-grounded models of heat exchange and metabolism can be integrated to meet these challenges. I will show how a formal metabolic theory – dynamic energy budget theory (developed by Kooijman) – can be combined with the biophysical models constituting the Niche Mapper package (developed by Porter). This integrated model, which I am implementing as an R package ('NicheMapR') can then be driven by high-resolution climate data to determine the acute (stress) and chronic (life history) responses of species to environmental gradients. Such a modelling framework provides unique opportunities for understanding the causes and consequences of different climatic adaptations at different spatial scales in the past, present and future.

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11:10 Sunday 1st July 2012

A9.3

The macrophysiology of marine organisms in a changing ocean: Latitudinal variation in the vulnerability to elevated temperature and CO_2 in the periwinkle *Littorina littorea*

Piero Calosi (Plymouth University, UK), Sedercor Melatunan (Plymouth University, UK), Jonathan J. Byrne (University of Birmingham, UK), Robert L. Davidson (University of Birmingham, UK), Mark R. Viant (University of Birmingham, UK), Steve Widdicombe (Plymouth Marine Laboratory, UK) and Simon Rundle (Plymouth University, UK)

Marine taxa appear to show a variety of ecological and physiological responses to elevated temperature or high pCO_2/low pH conditions, but only a few studies so far have considered the interactive effects of these environmental variables. In addition to this, virtually no information is available on how different populations of a same species living along an environmental gradient will respond to these multiple global change drivers.

Here we present results on the comparative and integrative ecology, physiology and metabolomics of six populations of the edible periwinkle *Littorina littorea* living across the North East Atlantic thermo-latitudinal gradient and exposed to elevated levels of temperature and CO_2. Our results support the idea that differential levels of vulnerability to future environmental scenarios observed in different populations of marine taxa can be characterized by a certain degree of genetic differentiation. Further, we show that existing levels of adaptation to current temperature regimes could influence taxa's future responses, including the future risk for geographical range shifts, migration and contractions, potentially underpinning differences in the potential for further adaptation.

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11:40 Sunday 1st July 2012

A9.4

Using hybrid zones to uncover the genetic basis of physiological variation along environmental gradients

Patricia M. Schulte (University of British Columbia [UBC], British Columbia, Canada), Jessica L. McKenzie (UBC, British Columbia, Canada), Anne C. Dalziel (UBC, British Columbia, Canada) and Timothy H. Vines (UBC, British Columbia, Canada)

Hybrid zones, where closely related taxa meet and interbreed, commonly occur along gradients of climatic and ecological variables, and are ideal systems in which to study the genetic basis of environmental adaptation. Here we discuss two examples of such systems: hybrid zones in killifish (*Fundulus heteroclitus*) and stickleback (*Gasterosteus aculeatus*).

Killifish demonstrate counter-gradient variation in metabolic rate, such that fish of the northern subspecies have a higher metabolic rate than fish of the southern subspecies when compared at a common temperature. Using hybrids between northern and southern subspecies of killifish, we have shown that variation in metabolic rate is strongly associated with mitochondrial, but not nuclear, genotype, and might be playing a role in thermal adaptation.

In stickleback, we have shown that non-migratory, fresh-water populations of three-spined stickleback have repeatedly evolved a decreased capacity for prolonged swimming compared to their migratory marine ancestors. Using F_1 and F_2 hybrid crosses, we found that this variation in exercise performance is associated with divergence in a variety of morphological and physiological traits. Examination of a natural hybrid zone in British Columbia suggests that there might be particularly strong selection on pectoral fin length, suggesting the importance of this trait in influencing exercise capacity along a gradient of stream flows.

Together, these examples demonstrate the power of using hybrid zones and controlled laboratory crosses to understand the genetic basis of physiological variation along environmental gradients.

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12:10 Sunday 1st July 2012

A9.5
Hypoxia tolerance is associated with a unique gene expression profile in intertidal sculpins

Jeffrey Richards (University of British Columbia, British Columbia, Canada), Milica Mandic (University of British Columbia, British Columbia, Canada), Marina Ramon (University of Southern California, California, USA) and Andrew Gracey (University of Southern California, California, USA)

In response to hypoxia exposure, an organism must modify a number of complex traits to enhance survival. Some of these traits are determined by the coordinated activities of hundreds of genes. Phenotypic responses to changes in the environment vary among species and in order to understand the evolutionary or ecological implications of this variation it is critical to elucidate the differences in large-scale patterns of gene expression across species.

Sculpins, a group of closely-related benthic fishes, live along the marine intertidal zone and show remarkable variation in hypoxia tolerance. We examined broad-scale patterns of gene expression using heterologous microarrays among six species of sculpin that were exposed to O_2 tensions at 65% of their respective P_{crit} for 72 hours. Results suggest that there is little change in transcription levels in hypoxia-tolerant species until at least 24 hours of exposure to hypoxia, whereas hypoxia-sensitive species exhibit early transient changes in gene expression followed by a return to baseline in the late stages of hypoxia.

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12:30 Sunday 1st July 2012

A9.6
Influence of latitudinal thermal gradients on rates of protein synthesis and metabolism in gammarid amphipods

Nia M. Whiteley (Bangor University, Wales, UK), Rose Crichton (Bangor University, Wales, UK), Sam P.S. Rastrick (University of Plymouth, UK)

In order to further understand the relationships between thermal gradients and the physiological capacities of aquatic ectotherms to compensate for environmental change, whole-animal fractional rates of protein synthesis (indicator of growth and thermal sensitivity) and rates of metabolism (total energy expenditure) were determined in four species of gammarid amphipod with differing latitudinal distribution patterns along the coasts of Western Europe (38–79°N). Measurements were taken from acclimatised amphipods from five latitudes under natural summer conditions of optimal food supply and temperature.

Metabolic rates standardized for body mass were generally higher in the temperate species (*Gammarus locusta* and *Gammarus duebeni*) at intermediate latitudes than in the arctic/boreal species (*Gammarus oceanicus* and *Gammarus setosus*) at polar latitudes (79ºN). Within species, responses varied as metabolic rates were conserved across latitudes in the temperate species, but fell in the sub-arctic/boreal species with latitude. A similar response was observed for protein synthesis rates, as latitude had no effect in temperate species. Two different responses, however, were observed at the polar latitude as synthesis rates were considerably lower in the sub-arctic/boreal species but similar to temperate values in the arctic species.

Collectively, the data demonstrate that compensatory capacities are well established in temperate, eurythermal gammarids but are limited in the sub-arctic/boreal species at polar latitudes. Surprisingly, protein synthesis rates might not be as limited in the permanent cold as previously suggested. Relationships between compensatory capacities of gammarids and phylogenetic relationships, latitude, thermal tolerances, acclimation ability, growth rates and vulnerabilities to further environmental change will be explored.

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12:50 Sunday 1st July 2012

A9.7
Soil invertebrates and climate change in hot and cold deserts

Diana Wall (Colorado State University, Colorado, USA)

Extreme terrestrial ecosystems such as the Antarctic (cold, dry) and temperate deserts (hot, dry) differ dramatically in plant productivity, decomposition rates and soil biodiversity. The species richness of soil invertebrates is lower in the cold desert (McMurdo Dry Valleys, Antarctica) compared to the hot desert (Chihuahuan Desert, New Mexico, USA), but both deserts have alkaline soils and a high heterogeneity of soil chemical and physical properties.

The responses of soil biota in these ecosystems to temperature and moisture can inform future scenarios of climate change. We compared the factors – climate, habitat suitability (i.e. soil chemical and physical properties), and biotic interactions (i.e. plants and other biota) – determining soil community structure, activity and functioning in these deserts. Experimental manipulations of temperature and moisture were imposed to measure potential responses of the soil communities to climate change.

Results from these experiments and observed warming events in the cold desert indicate that climate change in extreme desert ecosystems alters soil habitat suitability and has immediate impacts on the few soil species present and potentially their role in ecosystem functioning. These changes are less detectable in hot desert ecosystems of higher soil animal diversity.

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13:50 Sunday 1st July 2012

A9.8
No place to hide: Plants surviving the climate of Antarctica

Sharon A Robinson (University of Wollongong [UoW], Australia), Jane Wasley (Australian Antarctic Division, Australia), Laurence J. Clarke (UoW, Australia), Melinda J. Waterman (UoW, Australia), Diana H. King (UoW, Australia), Jessica Bramley-Alves (UoW, Australia), Quan Hua (Australian Nuclear Science and Technology Organisation, Australia) and Wolfgang Wanek (University of Vienna, Austria)

Antarctica is the coldest, driest, windiest continent on Earth and most vertebrates confine their visits to the summer breeding season. Plants, however, with their sedentary nature are forced to endure the worst of

the weather. Plant-life is dominated by mosses, with the two vascular species restricted to the peninsula. Availability of water is a key driver for the distribution of vegetation, with luxuriant turfs found along ephemeral melt-streams or around melt lakes. The relative plant tolerance to submergence or desiccation determines the location of the various moss species, making their distribution vulnerable to changing water availability.

Accurate dating of mosses using modern radiocarbon techniques, combined with stable isotope ($\delta^{13}C$) measurements to track water availability during growth, allows us to explore the influence of environmental variables on growth and provides a dramatic demonstration of the impact of changes in water availability through space and time. δ13C profiles at many sites indicate a drying trend over recent decades and this is associated with reduced moss growth rates. This recent drought is superimposed on landscape level drying due to regional uplift since deglaciation, which results in former moss beds being overgrown by more drought-tolerant lichens. The finding that $\delta^{13}C$ signals laid down as the mosses grow can be used to determine changes in microhabitat water availability means that in future Antarctic mosses could be used as proxies for past coastal climate.

Future changes in water availability might determine the fate of these mosses and the associated microflora and fauna communities that form oases of biodiversity.

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14:30 Sunday 1st July 2012

A9.9

Variability of ecomorphological and metabolic traits in plant species across abiotic gradients in the Kerguelen Islands (sub-Antarctic)

Françoise Hennion (Université de Rennes 1, France), Marie Hermant (Université de Rennes 1 and Université des Sciences et Technologies de Lille 1, France), Philippe Vernon (UMR 6553 Ecobio Station Biologique de Paimpont, France), Peter Convey (British Antarctic Survey, UK), Andreas Prinzing (Université de Rennes 1, France and Alterra Centre for Ecosystem Studies, Netherlands)

Assessing potential responses of species to climate and other environmental changes will be critical for attempts to mitigate the effects on biodiversity. While studying their plastic and evolutionary responses is not easily tractable for whole sets of species, a useful approach is to explore the phenotypic variability of species in nature in close connection with environmental variation. Such studies, although not identifying the respective contributions of phenotypic and genotypic variation, can still be very informative by providing observations on the responses of organisms to real environments over large scales.

Abiotic gradients provide continuous series in nature for factors that trigger biological responses. Furthermore, comparing patterns of response between restricted range endemic species and more widely distributed or invasive species could help elucidate the importance of adaptation. We therefore studied the variability of traits in plant species across environmental gradients in the sub-Antarctic Kerguelen Islands. These islands are currently subject to rapid climate change and show strong abiotic gradients. They represent a simple system with a small flora.

We measured morphological, phenological, ecophysiological and metabolic traits. Along each gradient we measured water saturation, pH and conductivity in the soil and temperature. We tested the effects of environmental variables on trait values and calculated phenotypic integration and its variation. We found that phenotypic variation of ecomorphological traits across environmental gradients was significantly related to the endemism level of species to Kerguelen. Our initial data indicate that a large part of the biogenic amines present in plant leaves correlate with this relation.

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15:00 Sunday 1st July 2012

A9.10

Opening the black box: Mechanisms underpinning insect diapause and biological memory of environmental gradients

Scott A.L. Hayward (University of Birmingham, UK)

At temperate latitudes a specialized state of dormancy, termed diapause, is used as an overwintering strategy by virtually all insects. It is critical in regulating species phenology, distribution and survival. Despite the importance of diapause, we are only just beginning to understand the molecular mechanisms that underpin this adaptation to seasonal change.

The induction of diapause by changing environmental gradients (principally photoperiod and temperature) often occurs in the adult stage, while the initiation of dormancy is typically delayed until the subsequent generation. Thus, the programming of diapause requires a transgenerational component, but we know very little about how this 'biological memory' of environmental information is transferred. Bias-free metabolomic and lipidomic screens provide an excellent tool to identify the major pathways involved in regulating diapause, and to investigate whether the biological memory of diapause programming is characterized by a distinct metabolic signature. The specific role of different metabolites can then be further investigated through the use of dietary supplementation. Here I discuss core mechanisms underpinning diapause in the blue bottle, *Calliphora vicina*, and how this new information allows us to potentially manipulate insect overwintering and more effectively model species resilience to climate change.

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15:45 Sunday 1st July 2012

A9.11

How phasmids adapt to life in New Zealand: Physiological adaptation in a radiation of non-model organisms

Brent J. Sinclair (University of Western Ontario, Ontario, Canada), Alice B. Dennis (Landcare Research and Allan Wilson Centre, New Zealand), Luke T. Dunning (Landcare Research and Allan Wilson Centre, New Zealand) and Thomas R. Buckley (Landcare Research and Allan Wilson Centre, New Zealand)

Temperature is a key determinant of ectotherms' ability to occupy the breadth of environmental gradients, yet the mechanisms causing this limitation, influence of underlying biochemical constraints and evolutionary responses to gradients are poorly known. New Zealand stick insects have radiated from their tropical origins to inhabit a range of environments, including several independent radiations into cool temperate and sub-alpine environments. We have used a combination of physiological studies and high-throughput RNA sequencing to identify candidate processes that underlie thermal adaptation in nine clades. First, we identified significant interspecific variation in cold tolerance strategy – including one species that can withstand internal ice formation. Second, to investigate the mechanisms underlying the transition to freeze tolerance, we compared transcriptional responses to cold shock across the phylogeny using high-throughput RNA sequencing. Third, we identify signatures of selection in the nucleotide sequences of glycolytic enzymes for all of the New Zealand phasmid genera in comparison with their outgroups from New Caledonia and Australia, revealing adaptation in energy metabolism associated with the transition from tropical to temperate climates. Together this allows us to develop a picture of the molecular and physiological changes that occur with radiation into cold environments, and thus helps us better identify the evolutionary pressures that occur along climatic gradients.

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16:15 Sunday 1st July 2012

A9.12
Life history variation along the temperature and altitudinal gradients in the common vole (*Microtus arvalis*)

Mikko Lehto Hürlimann (University of Lausanne, Switzerland), Laure Figeat (University of Lausanne, Switzerland) and Pierre Bize (University of Lausanne, Switzerland)

Many studies have documented variation in mammal physiology and life histories along altitudinal gradients, yet critical gaps in our knowledge include whether those differences reflect plastic responses to the environment or genetic adaptation, and what the nature is of the factors driving these changes in physiology and life histories. Using a common garden experiment, I will show in a small rodent, the common vole (Microtus arvalis), that high altitude individuals have a slower pace of life than low altitude individuals (i.e. they are producing fewer pups that are growing at slower rates). High-altitude individuals were also found to have higher non-shivering thermogenesis (NST) capacity than low-altitude individuals. Thus, this common garden experiment provides strong evidence for genetically-based variation in the pace of life and physiology along an altitudinal gradient.

Positive selection on NST in high altitude voles is likely to divert resources from growth and reproduction into heat production. Hence, I will also present preliminary results from an artificial selection experiment on NST testing whether those changes in life histories along an altitudinal gradient might be mediated via exposition to cooler temperature and greater NST capacity. In agreement with this hypothesis, preliminary results show that voles in the high NST lines produce pups with a slower growth rates compared to low NST voles.

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16:35 Sunday 1st July 2012

A9.13
Effects of ambient cadmium with calcium on mRNA expressions of calcium uptake-related transporters in zebrafish (Danio rerio) larvae

Su Mei Wu (National Chiayi University, Taiwan, R.O.C.), Ming-Yi Chou (RIKEN Brain Science Institute, Japan) and Chia-Hao Lin (Institute of Cellular and Organismic Biology, Academia Sinica, Taiwan, R.O.C.)

The mRNA expression levels of Ca^{2+} transporter genes, including the epithelial calcium channel, sodium/calcium exchanger 1b (NCX1b) and plasma membrane calcium ATPase 2 (PMCA2) were measured in zebrafish larvae after exposure to 0.08 μM Cd^{2+} in water mixed with 0.2 mM Ca^{2+} (low-Ca) or 2 mM Ca^{2+} (high-Ca). The gene expression levels for ECaC and NCX1b decreased at the 48 and 72-hour mark, respectively; however PMCA2 transcript levels only decreased 96 hours after exposure to Cd2+ in a low-calcium environment. The epithelial calcium channel transcript levels of larvae were not affected; however, the expression levels of PMCA2 transcripts were increased at 72 hours, while the expression levels of NCX1b transcripts significantly decreased at 48 and 96 hours after exposure to Cd^{2+} in a high-calcium environment. The Ca^{2+} contents of larvae significantly decreased after Cd^{2+} exposure in a low-calcium environment, however the Ca^{2+} contents were evidently higher after exposure to Cd^{2+} in a high-calcium environment, except for the 48-hour mark. Taken together, our results show that the Ca^{2+} transporters of zebrafish larvae were impacted after exposures of 0.08 μM Cd. In the exposure condition of 0.08 μM Cd, however, the epithelial calcium channel- and PMCA2-transcripts could be restored to control levels after the fish were treated in an environment with high calcium.

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Poster Session – Sunday 1st July 2012

A9.14
Glycogen metabolism in the gills and hepatic tissue of tilapia (*Oreochromis mossambicus*) during short-term cadmiu exposure

Shu-Chuan Tsai (Central Taiwan University of Science and Technology, Taiwan, R.O.C.), Yu-Siang Lin (National Chiayi University, Taiwan, R.O.C.), Hui-Chen Lin (Tunghai University, Taiwan, R.O.C.) and Su Mei Wu (National Chiayi University, Taiwan, R.O.C.)

Glycogen metabolism is the principal energy source in both vertebrates and invertebrates during responses to environmental fluctuations and stress. Glucose is released through the degradation of glycogen by glycogen phosphorylase, and energy is mainly supplied by the oxidation of glucose and lactate as a result of carbohydrate metabolism. Heavy metals, for example cadmium (Cd), exert a wide range of pathological effects on fish. Stress responses upon Cd exposure include increases in metallothionein expression and cortisol levels that all need use up energy. Cortisol could influence ion balance regulation, enhancement of Na^+/K^+-ATPase activity and induce energy metabolism during stress.

Male tilapia larvae were exposed to 44.45 μM ambient Cd for 12 hours and their blood glucose significantly increased but their lactate levels were not significantly different. The glycogen phosphorylase activity increased immediately after 0.75 to 3.00 hours of Cd exposure in the gills, and after one to six hours in the liver, respectively. Plasma cortisol level increased from 0.25 to 1.00 hour and recovered after three hours. Glucocorticoid receptor (GR) mRNA expression decreased after 0.75 hour of Cd exposure in the gills, but significantly increased after six hours in the liver. Ca^{2+}, Na^+, Cl^- and K^+ significantly decreased upon Cd exposure within six hours of Cd-induced toxic stress. The results suggest that cortisol spontaneously stimulates glycogen metabolism in the gills, and triggers a subsequent energy supply later in the liver.

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Poster Session – Sunday 1st July 2012

A9.15
The role of modified expression of *hmg1* gene in resistance of transgenic tobacco plants to copper ions

Alexander Ermoshin (Ural Federal University, Russia), Maria Maleva (Ural Federal University, Russia), Nadezhda Chukina (Ural Federal University, Russia) and Valeriya Alekseeva (Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Russia)

The amount of photosynthetic pigments was determined in transgenic tobacco plants with heterologous *hmg1* gene in different orientations towards the CaMV 35SS promoter under the oxidative stress caused by copper ions. The *hmg1* gene plays a key role in the biosynthesis of isoprenoid compounds in plant cytoplasm. It was shown that in tobacco plants with a higher level of *hmg1* gene expression, photosynthetic pigments are less damaged under stress compared to control plants; whereas in transgenic lines with a reverse form of *hmg1* gene the destruction of pigments increased. These data indicate that some isoprenoid compounds are involved in protection of the pigment complex from oxidative stress caused by copper ions in plant cells. The results could be used for the construction of plants tolerant to heavy metals.

The work was supported by the Federal Program of Ministry of Education and Science of Russia (projects № 14.740.11.1032 and P1301).

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Poster Session – Sunday 1st July 2012

A9.16

Physiological variation and proteomic modification in the gills of air-breathing fish, *Trichigaster microlepis*, after exposure to hypoxia

Hui-Chen Lin (Tunghai University, Taiwan, R.O.C.), Chun-Yen Huang (National Taiwan Normal University, Taiwan, R.O.C.) and Cheng-Huang Lin (National Taiwan Normal University, Taiwan, R.O.C.)

All species in the Anabantoidei suborder are aquatic air-breathing fish. These species have an accessory air-breathing organ, the labyrinth organ, in the branchial cavity and can engulf air at the surface to assist in gas exchange. *Trichogaster microlepis* has arterio-arterial shunts in the fourth gill arch specialized for the transport of oxygenated blood. The anterior (first and second) and the posterior (third and fourth) gills differ in the activity and relative abundance of Na^+/K^+-ATPase (NKA).

A two-dimensional gel electrophoresis was applied to compare the protein expression between the tissues from the anterior and posterior gill arches in *T. microlepis*. In hypoxic condition, the degree to which the fish responses to balance O_2 and metabolism requirements was also examined. Fish can change from aerobic to anaerobic respiration by utilizing glycogen as the fuel for glycogenolysis and can reduce NKA expression to further preserve energy consumption. Furthermore, the antioxidant ability helps fish to resist free radical damage in hypoxia.

Our results indicate that NKA increased in relative abundance at 1.5 and 5.0 hours, while carbonic anhydrase II did not change expression in the anterior gills after hypoxic exposure. Glycogen phosphorylase increased in relative abundance and the glycogen content decreased after three hours of hypoxic exposure. The antioxidant abilities in hypoxia increased in gills at three hours. From these results it can be seen that *T. microlepis* responds to hypoxic stress by changing ionic regulation, metabolism and antioxidation. Combined with proteomic results, the histological and functional approaches provide an integrated basis for investigating the multiple roles of the gill in aquatic air-breathing fish.

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Poster Session – Sunday 1st July 2012

A9.17

Metabolic rate reaction norm to temperature on intertidal crustaceans along a latitudinal gradient

Marco A. Lardies (Universidad Adolfo Ibañez, Chile), Tania Opitz (Universidad Adolfo Ibañez, Chile), Nelson A. Lagos (Universidad Santo Tomas, Colombia) and Leonardo D. Bacigalupe (Universidad Austral de Chile, Chile)

Is widely recognized that phenotypic adaptation to environmental fluctuations must frequently occur by pre-existing plasticity, but few studies have considered the change in phenotypic plasticity among geographic populations of marine crustaceans in response to ocean warming projections.

We examined the geographic variation in sea surface temperature as a source of variation in phenotype and in the reaction norm of metabolic rate using a common approach in five contrasting populations of intertidal crustaceans (six species) along a latitudinal gradient of Chilean coast (~2,500 km). We found that:

• metabolic rate increased in populations living in higher latitudes;

• the plasticity of metabolic rate is higher in high-latitude populations when compared with populations of low latitude; and

• in five species studied, there was a significant link between the populations (latitude) and temperature on oxygen consumption.

In combination, these results indicate that acclimation ability might vary greatly among populations and that understanding such variation will be critical if we are to predict the impacts of global warming on intertidal crustaceans. Finally, the significant population (genotype) × environment interaction in the trait studied indicates that there is genetic variation for phenotypic plasticity in response to thermal environments.

FONDECYT 1110743.

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Poster Session – Sunday 1st July 2012

A9.18

Temperature in sensitive red blood cell oxygen affinity in Atlantic cod near their southern distribution limit

Samantha Barlow (University of Liverpool, UK), David Righton (Centre for Environment Fisheries, UK), Julian Metcalfe (Centre for Environment Fisheries, UK) and Michael Berenbrink (University of Liverpool, UK)

Studies on the effects of temperature on the oxygen transport systems of aquatic organisms are important for understanding their capacity to cope with changes in thermal environment. Elevated temperatures generally increase the metabolic rate and thereby the oxygen demand of aquatic ectotherms. Furthermore, rising temperatures usually reduce the oxygen affinity of vertebrate haemoglobins, which, in fishes, can impair full oxygen loading at the gills.

Atlantic cod (*Gadus morhua*) possess two major haemoglobin genotypes whose prevalence change in accordance with a temperature cline from the Barents Sea to the northern Bay of Biscay (80°–45º North). Studies on acute temperature-challenged red cells of White Sea cod (67° North) have predicted a partial, adaptive reduction in the temperature sensitivity of oxygen-binding affinity in the heterozygote haemoglobin phenotype that is most common at the warmer, southern distribution limit of cod. Here we test this on red cells of heterozygous Irish Sea cod (53º N).

Surprisingly, between 5°C and 20°C and at constant pH values between 7.9 and 7.4, we find complete temperature insensitivity of red cell oxygen binding. Given the strong Bohr effect found in this study ($\Delta\log P50/\Delta pH > 1.0$), we suggest that short-term excursions into warm, supposedly intolerable water conditions will affect red cell oxygen affinity primarily by associated changes in red cell pH rather than by changes in temperature. Thus, modelling of temperature effects on *in vivo* red cell oxygen transport needs to take into account haemoglobin genotype, differences between acute and chronic temperature changes, and red cell pH.

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Poster Session – Sunday 1st July 2012

A9.19

Stomatal responses of *Eucalyptus* sp. grown at different concentrations of carbon dioxide and temperature in Western Sydney, Australia

Thomas Berg Hasper (University of Gothenburg, Sweden), David Ellsworth (Institute for the Environment [IfE], University of Western Sydney, Australia), Kristine Crous (IfE, University of Western Sydney, Australia), Audrey Quentin (IfE, University of Western Sydney, Australia) and Johan Uddling (University of Gothenburg, Sweden)

Atmospheric carbon dioxide (CO_2) increase is a major concern for areas of global forests. CO_2 is known to influence plants' physiological functions by reducing the stomatal conductance (g_s) and possibly affecting forest water balance. The objective of this study was to investigate the effect of growth under elevated CO_2 concentration ($[CO_2]$) and/or temperature on the primary short-term stomatal CO_2 response, stomatal dimensions and hydraulic conductance in *Eucalyptus globulus* grown in whole-tree chambers.

In order to compare g_s CO_2 responses among treatments, leaves from *E. globulus* trees were exposed to four levels of [CO_2] until g_s stabilization. Although leaves in all treatments exhibited stomatal closure responses to increased [CO_2], this response was significantly reduced in leaves growing in elevated [CO_2], showing that stomata of *E. globulus* do acclimate to growth [CO_2]. The stomatal CO_2 response of plants grown in elevated temperature did not differ from that in plants grown in ambient temperature. Anatomical and hydraulic measurements were also analysed and there were no significant effects of CO_2 or temperature treatment on stomatal density or size or hydraulic conductance.

In order to preserve plant species and their ecosystems, it is crucial to understand the impacts of higher [CO_2] and temperatures of a near-future climate on plant water use. This study indicates that while stomatal CO_2 responses have the potential to cause water savings under elevated [CO_2], this potential is reduced by stomatal acclimation to prevailing growth [CO_2] and is unlikely to be present during conditions when g_s is constrained by plant hydraulics.

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Poster Session – Sunday 1st July 2012

A9.20
Thermal dependence of developmental rate and energy use by embryonic geckos (*Paroedura picta*)

Zuzana Starostova (Charles University, Czech Republic), Michael J. Angilletta (Arizona State University, Arizona, USA), Lukas Kubicka (Charles University, Czech Republic) and Lukas Kratochvil (Charles University, Czech Republic)

In ectotherms, environmental temperature is the most prominent abiotic factor that modulates life-history traits. Despite this, we often do not know the proximate mechanisms that underlie the thermal reaction norms of key traits, such as body size. Here, we report the effects of three constant temperatures (24, 27 and 30°C) on the rate of embryonic development, the energetic cost of incubation and body size at hatching in the Madagascar ground gecko (*Paroedura picta*).

The cost of incubation was estimated as the difference between the energy content of an egg and energy content of the hatchling that emerged from that egg. As expected, the duration of incubation depended strongly on incubation temperature, with mean incubation periods being 107 days at 24ºC, 68 days at 27°C and 51 days at 30ºC. Hatchlings from eggs incubated at 24°C were significantly smaller than those from eggs incubated 27ºC or 30°C. Interestingly, the energy density of hatchlings was significantly lower at 24ºC in comparison to 27 and 30ºC.

Based on our estimates of the energetic contents of eggs and hatchlings, the energetic cost of incubation at 24ºC exceeded the cost at other higher temperatures. Therefore, the difference in body size at hatching resulted from a difference in the way that embryos used energy.

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Poster Session – Sunday 1st July 2012

A9.21
Metabolic scope in an indigenous and an invasive fish species: The effect of temperature

Paolo Domenici (CNR IAMC, Italy), Stefano Marras (CNR IAMC, Italy), Fabio Antognarelli (CNR IAMC, Italy), Michel Bariche (American University of Beirut, Lebanon) and Ernesto Azzurro (ISPRA, Italy)

The 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 an 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 metabolic performance. The increasing success of these species might be partly related to the fact that their optimal temperature, in terms of maximizing metabolic scope, is higher than that of indigenous ones. Within this context, our objective is to determine the effect of temperature on the metabolic scope of two fish that occupy a similar ecological niche: the native *Sarpa salpa*, and the invasive *Siganus rivulatus*, which has entered the Mediterranean from the Red Sea through the Suez Canal. This latter fish has already colonized large areas of the basin, especially its Eastern sectors, were it has probably misplaced the indigenous *S. salpa*. Our experimental results will provide insights on the adaptive potential of this invader to the Mediterranean Sea and on the possible competition with its native counterpart.

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Poster Session – Sunday 1st July 2012

A9.22
Does food deprivation-induced metabolic depression affect the hypoxia tolerance of juvenile rainbow trout (*Oncorhynchus mykiss*)?

Yuxiang Wang (Queen's University, Ontario, Canada), Yiping Luo (Queen's University, Ontario, Canada and Southwest University of China, China) and Scott MacIntyre (Queen's University, Ontario, Canada)

Rainbow trout, a keystone species occupying a temperate aquatic ecosystem, is fast-swimming and hypoxia intolerant. This species is of particular concern when studying the effects of hypoxia on an organism's physiological functioning. To determine the effect that metabolic depression induced by chronic food deprivation had on hypoxia tolerance, we deprived juvenile rainbow trout of food for five weeks during which we kept them at 15ºC. Each week, routine metabolic rate (RMR) and critical oxygen tension (P_{crit}) were measured. Resting and post-hypoxia fish (eight hours at ~50% air saturation) were sampled at the same time to measure metabolites in blood, liver and muscle as well as metabolic enzyme activities in select tissues. RMR and Pcrit significantly decreased following two and four weeks' of food deprivation, respectively, indicating that metabolic depression induced by food deprivation might confer an increased tolerance to low environmental [O_2]. Another indicator of hypoxia tolerance, marginal metabolic scope, however, did not change in response to metabolic depression, indicating that metabolic scope under hypoxic conditions was not changing. Furthermore, both food deprivation and moderate hypoxia elicited significant alterations in metabolites and enzymes activities, which suggested that the physiological state induced by these stressors might influence the organism's scope of survival during more severe acute bouts of hypoxia.

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Poster Session – Sunday 1st July 2012

A10 – SENSORY SYSTEMS: PHYSIOLOGY AND BEHAVIOUR

A10.1
Chemical cues that mediate predator–prey interactions in freshwater littoral fishes

Brian D. Wisenden (Minnesota State University, Minnesota, USA)

Small fishes in the littoral zone of freshwater systems fall prey to many predators. When grasped by a predator, tissues and cells of the prey are injured, causing the passive release of chemical compounds that are released in no other context. Near-by prey use these chemical cues to assess the risk of predation.

In this presentation I review quantitative studies conducted in the field that demonstrate how free-swimming field populations of fish respond to chemical alarm cues to adjust area use, the active time and active space associated with cue release, risk-sensitive information gathering, and field evidence for an immune function for the epidermal club cells of ostariophysan (minnows and kin) and percid fishes that have erroneously been linked to alarm cue function in the past.

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10:30 Saturday 30th June 2012

A10.2
Targeted metal effects on olfactory sensory neuron subpopulations in fishes

Greg G. Pyle (Lakehead University, Ontario, Canada) and William A. Dew (Lakehead University, Ontario, Canada)

The fish olfactory rosette is covered in a neuroepithelium comprising several cell types, including three sub-types of olfactory sensory neurons (OSNs). Ciliated cells (OSNs) are sensitive to bile salts, such as taurocholic acid (TCA), which can represent the presence of a predator. Microvillus cells are sensitive to amino acids, which can represent food. Crypt cells are sensitive to sex pheromones, which are important in reproduction.

The molecular signal transduction cascade is different between ciliated and microvillus cells, allowing us to use pharmaceutical interventions to either block or stimulate measurable OSN activity (using electro-olfactography) and demonstrate chemosensory specificity to standard cues (e.g. TCA or L-alanine). Once this specificity was established, we exposed fish to increasing, but environmentally-realistic concentrations of copper or nickel for 48 hours and retested their response to TCA or L-alanine. Copper exposure yielded a general chemosensory inhibition at high concentrations, but at lower concentrations the effects were restricted to ciliated cells. Nickel, however, exerted its toxic effects specifically on microvillus cells at all concentrations tested.

By understanding contaminant-targeting of OSN subpopulations, we can begin to predict behavioural deficits in contaminated environments and improve our ability to evaluate the ecological risk to resident biota.

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11:00 Saturday 30th June 2012

A10.3
Olfactory transduction in fish: Do fish smell the same way as mammals?

Mar Huertas (Centro de Ciências do Mar, Portugal and Michigan State University, Michigan, USA), Adelino V.M. Canario (Centro de Ciências do Mar, Portugal) and Peter C. Hubbard (Centro de Ciências do Mar, Portugal)

Olfactory receptors in fish are poorly described in comparison with those of mammals and the key elements of the transduction pathways are more varied, and can be different. So far, molecular data from several fish species indicate similarity with some parts of the mammalian olfactory receptor repertoire. Functional studies using mammalian agonists also suggest receptors with similar ligand binding sites. Despite, functional studies using known inhibitors of the two main olfactory transduction pathways in mammals, adenylate cyclase/cyclic-AMP and phospholipase $_C$*inositol* triphosphate (SQ 22356 and U73122, respectively) in the goldfish and European eel show contradictory results.

Surprisingly, increasing concentrations of 10^{-10} M to 10^{-6} M of U7322 never completely inhibited olfactory responses – as assessed by the electro-olfactogram (EOG) – to amino acids in goldfish or eels. At 10^{-6} M, however, U73122 caused significant reductions in EOG responses to amino acids and bile acids in the eel, but only to amino acids in the goldfish; bile acid responses were largely unaffected. Conversely, SQ22356 (up to 10^{-5} M) has little effect on bile acid or amino acid responses in the eel or goldfish. Moreover, experiments with l-*cis*-diltiazem, a selective inhibitor of cyclic nucleotide-gated channels and therefore olfactory transduction in mammals, failed to inhibit EOG responses in either species. Thus, inhibition by drugs commonly used in mammals depends on both the species and odorant in fish. This suggests that, even if fish olfactory receptors share a similarity with mammalian receptors, the olfactory transduction pathways differ from those of mammals and remain to be understood.

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11:20 Saturday 30th June 2012

A10.4
Brain activity and breathing patterns in the cockroach *Naupoheta cinerea*

Philip G. D. Matthews (University of Queensland, Australia) and Craig R. White (University of Queensland, Australia)

Insects display a wide range of gas exchange patterns, from continuous and cyclic patterns, through to highly episodic breathing patterns typified by long breath-hold periods between brief bouts of ventilation. The causes and consequences of these discontinuous gas exchange cycles (DGCs) have been the subject of much speculation.

DGCs tend to be displayed during periods of inactivity and low metabolic rate (MR). From this it has been hypothesized that DGCs arise as a consequence of low MR. Neurological changes associated with periods of inactivity, however, might also contribute to the emergence of this breathing pattern.

To test this theory, cockroaches were restrained within a respirometry chamber with their heads in contact with a thermoelectrically-controlled cold probe. This allowed their brains (cephalic ganglia) to be inactivated by cooling while simultaneously measuring their breathing pattern and MR.

We found that chilling the brain of a cockroach caused it to switch from a continuous to a discontinuous gas exchange pattern, while rewarming the brain caused it to revert to a continuous pattern. There was no difference between the MRs of cockroaches displaying either DGCs or continuous gas exchange. This suggests that while continuous and discontinuous gas exchange patterns are equally effective at satisfying the respiratory demand of a resting insect, DGCs arise due to the down-regulation of activity in the insect's brain, and not simply in response to low MR.

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11:40 Saturday 30th June 2012

A10.5
How do bottlenose dolphins react to identity information in the whistles of conspecifics?

Vincent Janik (University of St Andrews, Scotland, UK)

Bottlenose dolphins (*Tursiops truncatus*) are unusual in their identification signals in that each animal invents its own unique frequency modulation pattern for recognition. These signals are called signature whistles and their development is strongly influenced by vocal learning. Playback experiments have shown that the modulation pattern invented carries identity information for receivers.

Vocal learning also enables dolphins to imitate the signature whistles of conspecifics. Such imitation could be advantageous and help in addressing individuals or it could make the bottlenose dolphin recognition system less reliable because signature whistles do not always indicate who is calling. In my laboratory, we investigated this apparent contradiction by analysing whether:

- signature whistles can be distinguished from non-signature whistles;
- dolphin whistles have general voice features that can be used for the recognition of identity, heritage, age or sex;
- dolphins react to signature whistles in the same way as they do to non-signatures; and
- dolphins react to copies in the same way as they do to originals.

The results suggest that signature whistles are truly arbitrary signals that do not carry information on identity beyond the invented frequency modulation pattern. Furthermore, individuals react distinctively to being copied and can be addressed with their whistles. Copies produced in the wild are however recognizable as copies through systematic parameter alterations that are introduced by the copier. Thus, bottlenose dolphin signature whistles are reliable indicators of identity and effective labels for addressing conspecifics.

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12:00 Saturday 30th June 2012

A10.6
Biosynthesis, function and evolution of a male mating pheromone in the sea lamprey

Weiming Li (Michigan State University [MSU], Michigan, USA), Ke LI (MSU, Michigan, USA), Cory Brant (MSU, Michigan, USA), Michael Siefkes (Great Lakes Fishery Commission, Michigan, USA), Erin Walaszczyk (MSU, Michigan, USA), Huiyong Wang (MSU, Michigan, USA), Tyler Buchinger (MSU, Michigan, USA), Nicholas Johnson (US Geological Survey, Michigan, USA) and Yu-wen Chung-Davidson (MSU, Michigan, USA)

Fish pheromones are chemical cues that modify behavioural, physiological or developmental processes of individuals of the same species. Sexually-mature male sea lampreys (Petromyzon marinus) release mating pheromones that coordinate reproduction with ovulated females during their terminal life stage. Male sea lampreys, after becoming spermiated, actively release a bile acid, 7α,12α,24-trihydroxy-5α-cholan-3-one-24-sulfate (3kPZS) across their gills through specialized glandular cells. Ovulated female sea lampreys respond to synthesized 3kPZS at concentrations as low as 10^{-13} M by swimming directly upstream to the source.

Recently, we have integrated multidisciplinary approaches to examine the diversity of molecular structures and functions of this male pheromone system. Biochemical and molecular analyses indicated that 3kPZS is mainly synthesized in the liver and modified at the gills before secretion into the water. Chemical fractionation, coupled with behavioural assays, demonstrated that compounds with structures similar to 3kPZS likely function as minor components of the male mating pheromone. Physiological and chemical analyses showed that the male pheromone modifies the reproductive neuroendocrine system and progress of sexual maturation in both male and female sea lampreys. Furthermore, our results documented that male pheromone exposure suppresses the nocturnal locomotor rhythm in ovulatory females and affects melatonin production as well as the expression of several clock genes in the pineal complex and the brain. Comparative studies have suggested that 3kPZS signalling is probably a conserved system among petromyzonid species.

13:20 Saturday 30th June 2012

A10.7
The search for dominance pheromones in the Mozambique tilapia

Joao L. Saraiva (CCMAR, Centre of Marine Sciences, Portugal), Tina Keller-Costa (CCMAR, Centre of Marine Sciences, Portugal), Peter C. Hubbard (CCMAR, Centre of Marine Sciences, Portugal), Eduardo N. Barata (University of Évora, Portugal) and Adelino V. Canário (CCMAR, Centre of Marine Sciences, Portugal)

Chemical communication is a widespread phenomenon in the animal world but, in teleosts, very few pheromones have been chemically identified. In this work we present a bioassay to test synthetic analogues of a putative dominance pheromone in the Mozambique tilapia *Oreochromis mossambicus.*

Social groups of five females and five males were observed to assess dominance hierarchies. Urine was collected from the dominant males and used as a stimulus. A mixture of two olfactory active steroid isomers that were recently identified in dominant male urine was also tested as a putative dominance pheromone.

Experimental males were randomly taken from stock tanks and each went through a gradual process of social isolation. On the experimental day, a partition concealing a mirror was lifted in the isolation tanks and fish were observed interacting with their mirror image. Forty per cent of the males tested responded to their mirror image and only these animals received the following olfactory stimuli during mirror exposure: a) control water, b) dominant male urine and c) synthetic steroid isomers.

While males stimulated with dominant male urine attack less than control males, the synthetic steroid produces a highly variable response that does not statistically differ from the other stimuli. The results suggest that the complete dominance pheromone in male urine does not only consist of these two steroid isomers, but includes other – as yet unknown – compounds.

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13:50 Saturday 30th June 2012

A10.8
Detection of chemical irritants and carbon dioxide in rainbow trout

Lynne U. Sneddon (University of Chester, UK) and Catherine R. McCrohan (University of Manchester, UK)

Anthropogenic inputs into water bodies and rising carbon dioxide levels present potential dangers for aquatic animals, especially those chemicals with acidic properties. Whether fish detect these chemicals or whether these chemicals act as irritants is currently underexplored. Trigeminally innervated, mechanically sensitive chemoreceptors were previously identified on the head of the rainbow trout, *Oncorhynchus mykiss*, but it is not known whether these receptors are only responsive to noxious stimuli or have a general chemosensory function. This study aimed to characterize the stimulus–response properties of these receptors in comparison with polymodal nociceptors.

To determine which chemicals excite nociceptors, a range of chemical stimulants was applied to the mechanochemical receptors, including presumed non-noxious stimuli such as ammonium chloride, bile, sodium bicarbonate and alarm pheromone, and noxious agents such as acetic acid, carbon dioxide, low pH, citric acid, citric acid phosphate buffer and sodium chloride. Only noxious stimulation evoked a response, confirming their nociceptive function. All receptors tested responded to carbon dioxide in the form of mineral water or soda water. The majority of receptors tested responded to acidic chemicals.

Carbon dioxide receptors have been characterized in the orobranchial cavity and gill arches; however, this is the first time that external carbon dioxide receptors have been identified on the head of a fish. Since fish skin is in constant contact with the aqueous environment, acidic contaminants or hypercapnia might stimulate the nociceptive system in fish. This has welfare implications when the fish are unable to avoid such chemical stimuli.

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14:10 Saturday 30th June 2012

A10.9
Impact of light pollution on daily rhythms, behaviours and physiological functions of a non-human primate, *Microcebus murinus*

Thomas Le Tallec (Nationale Museum National d'Histoire [NMNH] and UMR 7179, France), Martine Perret (NMNH and UMR 7179, France) and Marc Théry (NMNH and UMR 7179, France)

Light pollution is a growing phenomenon to which wildlife is exposed, especially in urban and suburban areas. Its influence on the biological rhythms, behaviours and physiology of small nocturnal mammals has been described, but not exhaustively quantified.

The effect of light pollution has been studied in a non-human primate, the grey mouse lemur, *Microcebus murinus*, which is representative of small nocturnal mammals and strictly dependent on photoperiod. In a first experiment, eight animals were exposed for to light pollution characteristic of urban areas (high pressure sodium) 14 nights and compared to eight control animals exposed to near darkness (nocturnal luminosity). The animals were kept in individual cages under controlled conditions and fed *ad libitum*. We continuously recorded body temperature and locomotor activity by telemetry to study the daily rhythms. Locomotor and feeding behaviours were studied by video recording at the end of the experiment. Our results show that light pollution causes a phase shift (onset–offset) in daily rhythms and significantly affects body temperature patterns and locomotor behaviour.

In a second experiment, six animals were exposed to five weeks of light pollution (high-pressure sodium) and compared to six control animals exposed to near darkness (lunar luminosity). The daily rhythms, hormonal secretions (melatonin, cortisol and testosterone) and metabolism were studied by telemetry, ELISA assays and respirometry, respectively. The overall results demonstrate that light pollution affects daily rhythms, behaviours and physiological functions.

Our results suggest that light pollution in urban and suburban areas could be disadvantageous for small nocturnal mammals and could negatively affect populations.

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14:30 Saturday 30th June 2012

A10.10
How and why animals control and manipulate the polarization of light

Nicholas W. Roberts (University of Bristol, UK)

A remarkable diversity of biophotonic structures that control light exist in nature. Many animals use structural optics for visual signalling, different forms of camouflage and to improve their visual systems. Animals are not only able to control the flow of light, however; in several cases, they can control and manipulate the polarization of light as well.

Polarization describes the direction that light oscillates in and in this talk I will discuss two of our recent discoveries that demonstrate how bio-optical structures manipulate polarization.

The silvery reflections from pelagic fish, such as herring or sardine, help them background match their light environment. The reflectivity of typical multilayer structures reduces to half its maximum at particular angles of incidence but the silvery reflections from pelagic fish do not show this reduction in reflectivity. Our results demonstrate that by using multiple layers with three different optical properties, herring and sardine have an optical structure that does not polarize reflected light over all angles of incidence. This increases their reflectance so it is closer to optimal levels and improves their background-matching abilities.

Second, several species of stomatopod, commonly called mantis shrimps, have evolved linearly-polarizing structures for sexual signalling. We have discovered that the evolution of polarized signals in several species fits with a model of sensory bias. Our combined behavioural, trait and phylogenetic data suggest that models of sensory bias are not just applicable to the evolution of multi-component signals, but are more widely relevant to the evolution of signal multi-dimensionality.

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14:50 Saturday 30th June 2012

A10.11
From chemoreceptors to responses: CO_2 sensing in fish

Kathleen M. Gilmour (University of Ottawa, Ontario, Canada)

The existence in fish of CO_2-specific cardiorespiratory chemoreflexes was the subject of some debate for a number of years. However, work over the last decade has firmly established that CO_2-sensitive chemoreceptors are present in the gill, and that stimulation of these receptors activates ventilatory and cardiovascular responses such as hyperventilation, bradycardia and increases in systemic resistance. The branchial CO_2-sensitive chemoreceptors appear to respond preferentially to changes in water CO_2 levels as opposed to blood or water pH. Recent work has identified neuroepithelial cells as the gill CO_2 sensors; this work also points to inhibition of a background K^+ channel as the mechanism underlying chemoreceptor activation by CO_2. A persistent issue concerns the benefits of hyperventilatory responses to hypercapnia, particularly given that these responses exhibit substantial inter-specific variation.

A better understanding of behavioural responses to hypercapnia may provide insight into this question.

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15:40 Saturday 30th June 2012

A10.12
Characterization of putative oxygen chemoreceptors of bowfin (*Amia calva*) and their role in the hypoxic ventilatory response during sustained hypoxia

Cosima S Porteus (University of British Columbia, British Columbia, Canada), Patricia A. Wright (University of Guelph, Ontario, Canada) and William K. Milsom (University of British Columbia, British Columbia, Canada)

The fish literature is peppered with evidence of two types of putative oxygen chemoreceptors: internal receptors that sense changes in blood PO_2 and external receptors that sense changes in water PO_2, but the exact location and morphology of these cells remains unknown. Our goal was to identify these elusive chemoreceptors using a facultative air breather, the bowfin.

One group of fish were exposed to prolonged hypoxia (42 torr for seven days) and had access to the surface of the water to breathe (hypoxic but not hypoxaemic). Another group were exposed to the same hypoxic protocol but did not have access to the surface to breathe (hypoxic and hypoxaemic). A third group of fish were kept in normoxia (control). Following exposure, the fish were sacrificed, and their gills were immunolabelled for specific cell markers.

Based on location within the gill, cell morphology and immunopositivity for cellular markers, we identified five types of putative oxygen chemoreceptors, three of which were innervated and thus could be involved in a reflex response. Based on changes in cell sizes between the experimental groups, we hypothesize that type I cells are internal chemoreceptors, while type II and III are involved in the hypoxic response, although not necessarily as chemosensing cells.

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16:10 Saturday 30th June 2012

A10.13
Effects of postnatal development, temperature and the pons on respiratory rhythm and pattern generation in rat pups

Bill Milsom (University of British Columbia, British Columbia, Canada), Angelina Fong (Macquarie University, Australia) and Beth Zimmer (Ferris State University, Michigan, USA)

Episodic breathing is common in lower vertebrates as well as in some species of hibernating mammals. While it is rarely seen in euthermic mammals, we discovered that episodic breathing patterns were common (>60%) in *in vitro* pontomedullary-spinal cord preparations at 27ºC from rat pups on the day of birth (P0), but the occurrence of this breathing pattern declined with postnatal development. Chemical inhibition and physical removal of the pons eliminated the episodic breathing pattern at P0. Interestingly, episodic breathing patterns could be restored in older preparations (P2–P4) by decreasing the temperature (23ºC), with or without the pons. Our data suggest that episodic breathing is part of a continuum that appears when respiratory drive is low. In preparations held at 27ºC, with a continuous rhythm, an episodic rhythm could be produced by activating GABA receptors (100 µM GABA). In preparations with an episodic pattern, antagonism of opioid receptors by naloxone (1–5 µM) did not affect the episodic rhythm, while blockade of GABAA receptors by bicuculline (10 µM) converted the episodic rhythm to a continuous rhythm. In preparations held at 23ºC, however, bicuculline (10 µM) had the opposite effect, promoting episodicity. Together, these data suggest the mechanisms required for episodic rhythm generation are intrinsic to the medulla, and are modulated by postnatal development, temperature-sensitive mechanisms (such as transient receptor potential channels) and pontine factors.

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16:30 Saturday 30th June 2012

A10.14
Tracing olfactory circuits of innate responses

Maria Luísa Vasconcelos (Champalimaud Neuroscience Programme and Instituto Gulbenkian de Ciência, Portugal), Nlia Varela (Champalimaud Neuroscience Programme and Instituto Gulbenkian de Ciência, Portugal)

It has been extremely difficult to study a circuit beyond the early stages of sensory processing in complex organisms. *Drosophila melanogaster* is an attractive model system for understanding a circuit because flies exhibit complex behaviours that are controlled by a nervous system that is numerically five orders of magnitude simpler than that of vertebrates.

A simple innate behaviour described in the fruit fly is a robust avoidance to CO_2. CO_2 was identified as a component of the stress odorant that is released by stressed flies and elicits a robust avoidance response in test flies. Exposure of flies to CO_2 over a broad range of concentrations activates only a single identifiable glomerulus, the v glomerulus.

We are using laser-guided tracing of circuits to further investigate the circuit that translates CO_2 detection into an avoidance response. In the past, we have used this technique to trace the circuit of the male pheromone 11-*cis*-vaccenyl acetate, which is dimorphic between male and female flies.

We have traced the neurons that innervate the v glomerulus. Besides a large number of local neurons, we have observed four different projection neurons that bring us insight into how the odour might be processed. We are now working on tracing the third-order neurons.

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16:50 Saturday 30th June 2012

A10.15
Neural activation in winners and losers

Chinmaya Sadangi (Uppsala University, Sweden), Per Ove Thörnqvist (Uppsala University, Sweden), Lars Ebbesson (Uni Environment, Norway) and Svante Winberg (Uppsala University, Sweden)

Previous social experience is known to impact the competitive ability of an animal. The tendency of winners winning and losers loosing is still in continuity but the neuroendocrinology involved in these behavioural effects remains unknown. In the present experiment, juvenile rainbow trout (*Oncorhynchus mykiss*) were allowed to interact in pairs for five days for one hour per day whereas controls were allowed to see each for one hour per day through the transparent partition but were not allowed to interact. Large fish were not allowed to see small fish and *vice versa* before they were allowed to interact. At the end of five days the fish were then sacrificed for subsequent collection of blood plasma and brains.

Plasma cortisol levels and brain monoamines and monoamine metabolite levels were measured. In a subset of fish, the brain expression of early immediate genes, *EGR1* and *C-fos*, were analysed by *in situ* hybridization and quantitative polymerase chain reaction. Neural activation was indicated by the expression of *EGR1* and *C-fos*, regional brain monoaminergic activity and plasma cortisol were compared in winners, losers and controls.

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Poster Session – Saturday 30th June 2012

A10.16
Gene expression in the olfactory epithelium of the European eel, *Anguilla anguilla*

Allison M. Churcher (University of Algarve, Portugal), Weiming Li (Michigan State University, Michigan, USA), Scot Libants (Michigan State University, Michigan, USA), Alessandro Coppe (University of Padova, Italy), Lorenzo Zane (University of Padova, Italy), Adelino V.M. Canário (University of Algarve, Portugal) and Mar Huertas (Michigan State University, Michigan, USA)

The European eel *Anguilla anguilla* has a complex lifecycle that includes both freshwater and seawater migrations. While a few studies have addressed the role of chemoreception in eels, much remains to be understood about the molecular, cellular and physiological mechanisms involved in eel olfaction. Here, we begin to characterize the molecular components involved in olfactory signal transduction in *A. anguilla*. For this, RNA was isolated from the olfactory epithelium of animals from different life stages and used to construct Roche 454 and Illumina sequence libraries. We also used a combination of bioinformatics approaches to search available transcriptome data for *A. anguilla*.

Our preliminary results indicate that at least 18 odorant receptors from the rhodopsin-like GPCR family, 10 chemosensory receptors from the glutamate GPCR family, one type 1 vomeronasal receptor and other genes involved in olfactory signal transduction (e.g. cyclic nucleotide-gated channels, adenylate cyclase and phospholipase C) are transcribed in the *A. anguilla* olfactory epithelium.

The next step is to construct an assembly for the olfactory epithelium transcriptome and examine patterns of differential gene expression. We expect that this approach will help to identify receptors and other genes that facilitate the transition from saline to freshwater environments and will direct physiological investigations on eel communication by identifying genes involved in olfaction (e.g. odorant receptors).

This research was supported by the Portuguese Fundação para a Ciência e a Tecnologia.

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Poster Session – Saturday 30th June 2012

A10.17
Olfactory sensitivity to urinary steroids in the Mozambique tilapia suggests two distinct receptor mechanisms

Tina Keller-Costa (Centro de Ciências do Mar and University of Évora, Portugal), Peter C. Hubbard (Centro de Ciências do Mar, Portugal), Eduardo N. Barata (Centro de Ciências do Mar and University of Évora, Portugal) and Adelino V.M. Canário (Centro de Ciências do Mar, Portugal)

Male Mozambique tilapia, *Oreochromis mossambicus*, establishes dominance hierarchies and uses urine signals to attract females and mediate aggression between males. The olfactory organ of both sexes is highly sensitive to the urine of dominant males. One urinary fraction shows strong concentration-dependent olfactory activity and its compounds were recently identified as two isomeric 3-glucuronidated pregnanes, differing only by the stereochemistry of a free hydroxyl group. The objective of this study was to assess the olfactory potency and receptor specificity of synthetic analogues of these compounds and related sex-steroids using the electro-olfactogram.

The olfactory system of *O. mossambicus* was sensitive to both 3-glucuronidated pregnane isomers as well as their keto-precursor. Stimulation of the olfactory epithelium with increasing concentrations of these substances (10^{-11} M to 10^{-5} M) produced well-defined sigmoidal concentration–response curves. Cross-adaptation suggested that the three steroids share a similar olfactory receptor but with different affinities, as they have significantly different apparent EC_{50} values. In addition, a 3-glucuronidated androstane was found to share the same receptor. *O. mossambicus* that could also detect estradiol-3-glucuronide, but the form of the concentration–response curve was different from the aforementioned steroids, saturating at 10^{-9} M. The tilapia did not detect related unconjugated, sulphated steroids or 17- or 20-glucuronidated steroids.

Our results suggest that this species has two receptor mechanisms characterized by specificity for 3-glucuronidated pregnanes/androstanes (3G-1) and estradiol-3-glucuronide. At least the specialized 3G-1 receptor serves the detection of urinary steroid glucuronides, which are used as part of a pheromone system to advertise social status.

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Poster Session – Saturday 30th June 2012

A10.18
Effect of acute salinity changes in anosmic European eel: Is olfaction involved in the osmoregulatory process?

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European eels can rapidly adapt to acute changes in environmental salinity. The physiological mechanisms of osmoregulatory function are well studied but the primary sense that triggers the osmoregulatory response is still unknown. We hypothesize that olfaction could be involved.

To test this theory, we transferred anosmic freshwater-adapted eels to seawater and anosmic seawater-adapted eels to fresh water. Then, we measured changes in olfactory epithelia (OE) histology, plasma osmolarity and cortisol zero, six, 24 and 96 hours after transfer.

The OE of freshwater fish transferred to seawater showed structural changes after six hours with an increase in thickness of the lamellae and replacement of columnar by globular mucous cells. The OE of seawater fish transferred to freshwater showed a dramatic decrease of cell layers, loss of receptor cells in the tips of the lamella and increase in mucous cells. All anosmic fish showed atrophy and loss of pseudostratified structure of olfactory receptor neurones after 12 hours. Anosmic freshwater fish transferred to seawater tended to swim close to the surface; controls did not. Plasma osmolarity increased in freshwater fish transferred to seawater (297.2 ±20.0 to 412 ±19.0 mOsm) but decreased in seawater-adapted fish transferred to freshwater (323.4 ±13.0 to 299.4 ±10.0 mOsm). Osmolarity changes in anosmic fish were faster than in controls at six hours. However, no significant changes were observed in cortisol levels.

We suggest that anosmia blocks the instant detection of environmental salinity changes and therefore compromises osmoregulatory adaptation to these changes and could cause chronic stress or death.

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Poster Session – Saturday 30th June 2012

A11 – CLIMATE CHANGE PHYSIOLOGY: ENVIRONMENTAL VARIATIONS AND THE RESPONSES OF FREE-LIVING ANIMALS

A11.1
Ecophysiology of avian migration in the face of current global hazards

Marcel Klaassen (Deakin University, Australia and Netherlands Institute of Ecology, Netherlands), Bethany J Hoye (Deakin University, Australia and Netherlands Institute of Ecology, Netherlands), Bart A. Nolet (Netherlands Institute of Ecology, Netherlands) and William A. Buttemer (Deakin University, Australia)

Long-distance migratory birds are often considered extreme athletes, possessing a range of traits that approach the physiological limits of vertebrate design. In addition, their movements must be carefully timed to ensure they obtain resources of sufficient quantity and quality to satisfy their high energy needs. Migratory birds may therefore be particularly vulnerable to global change processes, which are projected to alter the quality and quantity of resource availability.

Long-distance flight requires high and sustained aerobic capacity, so even minor decreases in vitality can have large negative consequences for migrants. In light of this, we assess how current global change processes might affect the ability of birds to meet the physiological demands of migration, and suggest areas where avian physiologists can help to identify potential hazards.

Predicting the consequences of global change scenarios on migrant species requires:

• reconciliation of empirical and theoretical studies of avian flight physiology;

• an understanding of the effects of food quality, toxicants and disease on migrant performance; and

• mechanistic models that integrate abiotic and biotic factors to predict migratory behaviour.

Critically, a multidimensional concept of vitality would greatly facilitate evaluation of the impact of various global change processes on the population dynamics of migratory birds.

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10:30 Saturday 30th June 2012

A11.2
Seasonal acclimatization: From the organism to cellular membranes

Walter Arnold (Research Institute of Wildlife Ecology, University of Veterinary Medicine, Austria)

Hibernators and daily heterotherms cope with the challenge of cold exposure and nutritional bottlenecks by abandoning the energetically-costly maintenance of a high body temperature (T_b). We have found similar reactions in several 'non-hibernating' large endotherms. Although T_b changes in the body core were small, the changes in the body's periphery and the reduction of energy expenditure were substantial.

Operation at lower T_b requires biochemical changes in the phospholipid composition for maintaining membrane integrity, a phenomenon well-known from ectothermic organism. We found according remodelling of membranes in mammals, but with apparently specific roles for essential polyunsaturated fatty acids (PUFA) of the n-6 and n-3 series. An important function of the diet-independent incorporation of PUFAs into phospholipid might be the compensation for temperature (Arrhenius) effects on membrane-bound enzymes.

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11:10 Saturday 30th June 2012

A11.3
Multiscale climate variation and the activity energetics of small and large endotherms

Murray Humphries (McGill University, Quebec, Canada), Guillaume Larocque (McGill University, Quebec, Canada), Tom Jung (Yukon Environment, Yukon, Canada) and Emily Studd (McGill University, Quebec, Canada)

The ability of endotherms to maintain activity across an extreme range of climate conditions is well-recognized, but even if temperature-independent activity is possible, few would expect endotherm movement and activity patterns to be unaffected by temperature. The forms, causations and implications of temperature-dependent activity by endotherms, however, remain remarkably unexplored.

Biologging advances in animal ecology are rapidly providing new and better data on animal movements in the wild, which can be used to advance our understanding of temperature-dependent activity patterns in endotherms. We will present new biologging data and novel analytical approaches to explore the multiscale temperature-dependence of activity in free-ranging endotherms, ranging in size from 200-g red squirrels (*Tamiasciurus hudsonicus*) to 1,000-kg bison (*Bison bison athabascae*). We will focus on thermoregulatory influences on activity, including whether small and/or large endotherms are less active when exposed to air temperatures that are a long way outside their thermoneutral zone or whether they appear to use increased activity in the cold to generate the heat needed for thermoregulation.

We will conclude by considering the ecological implications of temperature-dependent endotherm activity in a climate-changed future.

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11:35 Saturday 30th June 2012

A11.4
Seasonal adaptations in high Arctic Svalbard rock ptarmigan

John Lees (University of Manchester, UK), Robert Nudds (University of Manchester, UK), Karl-Arne Stokkan (University of Tromso, Norway), Lars Folkow (University of Tromso, Norway) and Jonathan Codd (University of Manchester, UK)

The Svalbard rock ptarmigan, *Lagopus muta hyperborea*, experiences extreme photoperiodic and climatic conditions on the Arctic Archipelago of Svalbard. This species, however, is highly adapted to live in this harsh environment. One of the most striking adaptations found in these birds is the deposition, prior to onset of winter, of fat stores that can comprise up to 32% of body mass and are located primarily around the sternum and abdominal region. This fat, while crucial to the birds' survival, also presents a challenge in that the bird must maintain normal physiological

function with this additional mass. These stores are likely to constrain the respiratory system, as the sternum and pelvic region must be moved during ventilation and carrying this extra load can also impact upon the energetic cost of locomotion.

Here, we demonstrate that winter birds have a reduced cost of locomotion when compared to summer birds. This is a remarkable finding, given that during winter these birds have almost twice the body mass of those in summer. These results suggest that Svalbard ptarmigan are able to carry the additional winter fat without incurring any energetic cost. As energy conservation is paramount to these birds, minimizing the costs of moving around when resources are limited would appear to be a key adaptation crucial for their survival in the barren Arctic environment.

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12:00 Saturday 30th June 2012

A11.5
Turning up the heat: Investigating the physiological effects of climate change on mammalian herbivores

Patrice Kurnath (University of Utah, Utah, USA) and M. Denise Dearing (University of Utah, Utah, USA)

Mammalian herbivores are being impacted by global climate change. One potential mechanism to explain population declines in mammalian herbivores is temperature-dependent toxicity; i.e. dangerous plant secondary compounds (PSCs) are perceived by herbivores as more toxic at warmer ambient temperatures due to decreased liver metabolism. To test this hypothesis, we investigated how ambient temperature impacts liver metabolism in the herbivorous desert woodrat, *Neotoma lepida*, using hypnotic state assays (HSAs).

In a cross-over design, wild caught *N. lepida* (N=13) were housed for 30 days at two ambient temperatures (warm = 29°C and cool = 21ºC), after which HSAs were administered. Hexobarbital, a hypnotic agent recognized as a legitimate estimator of liver metabolism and proxy for PSC detoxification, was administered via an intraperitoneal injection (100 mg/kg). The time spent in a hypnotic state was measured for each woodrat. This sleep time is inversely proportional to liver function, with decreased liver function equating to longer sleep times.

The average sleep time of woodrats acclimated to the warm temperature was almost 50% longer than the average sleep time of cool-acclimated woodrats (paired t-test, $p<0.01$). These results demonstrate that warmer ambient temperatures adversely affect liver function, and might provide a physiological mechanism through which climate change acts on herbivorous mammals.

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12:15 Saturday 30th June 2012

A11.6
Cardiorespiratory tailoring among sockeye salmon population for their river migration and its relevance to conservation

Anthony (Tony) P. Farrell (University of British Columbia [UBC], British Columbia, Canada), Erika Eliason (UBC, British Columbia, Canada), Michael Donaldson (UBC, British Columbia, Canada), Kristi Miller (Fisheries and Oceans Canada, Ontario, Canada), Steve Cooke (Carleton University, Ontario, Canada) and Scott Hinch (UBC, British Columbia, Canada)

The return migration of sockeye salmon to spawn in the Fraser River, Canada, is rather magnificent. It involves large distances and hydraulic challenges that are negotiated in a short period of time. Moreover, the entire lifetime fitness of a sockeye salmon rests on a successful, once-in-a-lifetime river migration that is performed without eating. Salmon then spawn and die.

Our studies of the physiology and behaviour of river migration show that once sockeye salmon enter the Fraser River, they progress upstream with remarkably similar grounds speeds of 20–50 km day^{-1} despite encountering multiple and substantial hydraulic challenges.

Successful migration is aided by population-specific differences in aerobic scope, which appear to be tailored ahead of the migration to the particular hydraulic and temperature challenges are likely to be encountered when swimming to the spawning area. Population-specific variation in aerobic scope is supported by larger hearts with a better oxygen supply to the myocardium and more ventricular adrenoceptors, all of which point to a local adaptation as a result of fidelity to a particular spawning area and genetic isolation.

By combining tissue biopsies with salmon biotelemetry, we have related physiological status with salmon fate, including the consequence of sockeye salmon encountering fishers. Collectively, this work is being used by local management agencies in their efforts to conserve abundant sockeye salmon stocks in the Fraser River.

Supported by NSERC Canada and the Canada Research Chair program.

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13:30 Saturday 30th June 2012

A11.7
Heat dissipation limit theory and avian responses to global warming

David Gremillet (CEFE-CNRS, France) and Amelie Lescroel (Rennes University, France)

Organisms are considered as primarily limited by their capacity to acquire energy, and mechanistic models used to predict the impact of global warming on animal distribution and abundance largely comply with this paradigm. In great contrast, recent heat dissipation limit theory (HDLT) predicts that rates of energy expenditure might also be limited by the capacity of animals to shed metabolic heat when fed *ad libitum*.

The HDLT has so far mainly been developed and tested in captive rodents, and its implications for further taxonomic groups studied in the wild have to be evaluated. During this presentation, we will explore the implications of the HDLT for avian ecophysiology, in particular for the evolution of avian functional traits, such as body temperature, plumage colour, brood patch size and thermal insulation. Further, we will discuss and speculate upon the relevance of the HDLT for mechanistic models designed to forecast avian responses to global warming.

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14:10 Saturday 30th June 2012

A11.8
Seasonal variation of resting metabolic rate and body mass in free living weasels, *Mustela nivalis*

Paulina A Szafranska (Mammal Research Institute [MRI], Polish Academy of Sciences, Poland), Karol Zub (MRI, Polish Academy of Sciences, Poland) and Marek Konarzewski (MRI, Polish Academy of Sciences and University of Bialystok, Poland)

Seasonal changes of metabolic rates and body mass are major physiological responses of mammals to shifting ambient temperatures and food availability. At the population level, those responses can be due to the selective mortality/emigration of individuals characterized by specific trait value(s), or concerted changes of those traits at the individual

level. The latter implies a consistency of individual responses to changing seasonal conditions. We studied seasonal changes in body mass and resting metabolic rate (RMR) in free-ranging weasels (*Mustela nivalis*) to test the consistency of these traits in individuals caught in different seasons of the year.

While body mass was remarkably stable over the seasons, body mass-corrected RMR significantly varied and was lowest in winter. Within-individual, between-season repeatability was significant for both body mass (intraclass correlation $\tau = 0.59$, $p=0.0001$) and RMR ($\tau = 0.39$, $p=0.03$). We demonstrated that individuals characterized by above- or below-average body mass and RMR in one season also tend to maintain above-/below-average values of the traits studied in other seasons.

This is the first study to demonstrate that seasonal changes in RMR observed at the population level are in part due to a consistency in individual responses to shifting environmental conditions.

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14:35 Saturday 30th June 2012

A11.9
Sensitivity to thermal extremes predicts species distribution of Australian *Drosophila*

Johannes Overgaard (Aarhus University, Denmark), Ary A. Hoffmann (University of Melbourne, Australia) and Michael R. Kearney (University of Melbourne, Australia)

Climatic factors influence the distribution of ectotherms, raising the possibility that distributions will shift as rapid climate change occurs. Previous comparisons of performance curves for temperate and tropical species suggest that species from tropical areas are relatively more susceptible to climate change, but this prediction has not been tested when: related species are considered; acclimation effects are included; and local thermal extremes are incorporated into comparisons.

Furthermore, it is unknown whether performance curves and/or critical tolerance limits are useful proxies to model distributions in the first place.

In the present study, we obtained data from 10 *Drosophila* species with different latitudinal distribution along the Australian east coast and showed that the thermal dependence of fecundity, developmental success, developmental time and adult heat resistance was generally similar in tropical and widespread/temperate species groups. In contrast, substantial differences in adult cold responses persisted under all acclimation regimes. Using these estimates, we establish simple predictive models using historical environmental data from the Australian continent and the predictions modelled are calibrated against several hundred field observations of species distributions of the 10 species studied. Our study demonstrates the following points:

• Species-specific distributions are predicted by extreme tolerance and not performance curves.

• Acclimation responses need to be included to produce reliable predictions.

When exploring the putative distribution changes under a climate change scenario, we find that both widespread/temperate species and tropical species are faced with the risk of severe local extinctions and range shifts.

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14:50 Saturday 30th June 2012

A11.10
Use and integration of experimental, field and telemetric approaches to assist in defining conservation measures for threatened freshwater turtles

Craig E. Franklin (University of Queensland, Australia)

Taking an integrative approach through the use of experimental approaches, combined with detailed field work involving telemetry, has the potential to provide invaluable information on critical habitats required by species in the wild and in predicting/assessing the impacts of environmental change and habitat disruption. This talk will highlight the conservation benefits gained by having a detailed understanding of the ecophysiological characteristics of an animal and then combining this knowledge with spatial and temporal data obtained via telemetry from animals tracked in their natural environment.

Freshwater turtles are considered to be one of the more threatened vertebrate taxons. Species that obtain a significant proportion of their oxygen via aquatic respiration (bimodal-respiring turtles) are particularly prone to habitat change associated with anthropogenic impacts on river systems, such as the construction of weirs, barrages and dams that create impoundments that are hypoxic. Field studies have been conducted on bimodal-respiring turtles where time–depth recorders and acoustic/radio telemetry has provided detailed information on the behaviour, movement patterns and habitat requirements of species. These data have assisted in defining management plans and future conservation measures.

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15:05 Saturday 30th June 2012

A11.11
Physiological understanding and monitoring: Can it ensure aquaculture sustainability in the face of uncertainty?

Nick Elliott (CSIRO, Australia)

Aquaculture is the fastest growing primary industry (~7% per year), and is expected to meet the bulk of the additional 40million tonnes of aquatic food needed by 2030 to maintain the current global per capita consumption. As an industry it encompasses a broad range of species and environments (natural and farming). It includes the ranching and 'fattening' of wild animals, the collection of wild broodstock for captive spawning and on-growing, through to fully domesticated stock with managed and closed lifecycles; the one common theme being the aquatic environment, but even this is extremely variable.

Compared to terrestrial agriculture, aquaculture is a young industry with major biological, nutritional and environmental changes occurring. We are therefore at the early stage of understanding animals' responses to environmental pressures. These stressors have a significant influence on the performance of the animal, the quality of the final product for human consumption, and ultimately the sustainability of an industry. On top of these farming environmental pressures, is the growing literature on the uncertainties of the impacts and timing of climate change, with suggestions that some farmed species will not survive in certain locations. I consider:

• What then can physiological research and monitoring provide for farm managers and for the welfare of the animals?

• What can it do to influence adaptation strategies against the impacts of climate and other environmental change?

• And is climate change physiology a concern for the industry?

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15:40 Saturday 30th June 2012

A11.12
High temperature tolerance among Atlantic salmon families: Connecting hypoxia tolerance, ventricle size and myoglobin level

Katja Anttila (University of British Columbia [UBC], British Columbia, Canada), Rashpal S Dhillon (UBC, British Columbia, Canada), Anthony P. Farrell (UBC, British Columbia, Canada) and Patricia M. Schulte (UBC, British Columbia, Canada)

This research investigated the variation in temperature tolerance among families of Atlantic salmon. Moreover, by measuring critical thermal maximum (CT_{max}) and hypoxia tolerance of 42 families of Atlantic salmon, we evaluated the association between temperature tolerance and hypoxia tolerance, and investigated which organ and cellular level factors affected variability. Temperature tolerance varied significantly both within and between families, indicating that temperature tolerance might be at least partly genetically determined. Also, a significant positive correlation existed between CT_{max} and hypoxia tolerance at the family level (R^2=0.47; p<0.001). While previous studies have shown that fish with relatively larger ventricles have a greater circulatory capacity that could be advantageous for elevated oxygen demand at high temperatures, our results show for the first time that relative ventricle mass and cardiac myoglobin level are correlated positively with CT_{max} (R^2=0.21; p<0.01 and R^2=0.16; p<0.05, respectively). High myoglobin levels might either facilitate oxygen transfer from venous blood to spongy myocardium or enhance oxidative energy production capacity, which is currently under examination. Thus, our results suggest that maximum temperature tolerance is be partly heritable, which could be advantageous during global warming if fish can adapt fast enough for the rapid pace of changing temperatures. Individuals with relatively larger ventricles and higher cardiac myoglobin level might have better prospects in this case.

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16:05 Saturday 30th June 2012

A11.13

Cardiorespiratory and metabolic responses to hypoxia of growth hormone transgenic, diploid and triploid Atlantic salmon (*Salmo salar*) yolk sac alevins

Elias Polymeropoulos (University of Tasmania, Australia), Debbie Plouffe (AquaBounty, Prince Edward Island Canada, Canada), Sacha Leblanc (Mount Allison University, New Brunswick, Canada), Suzie Currie (Mount Allison University, New Brunswick, Canada), Nick Elliott (CSIRO, Australia) and Peter Frappell (University of Tasmania, Australia)

In Atlantic salmon, growth hormone (GH)-transgenesis leads to dramatically accelerated growth rates compared to non-transgenics. Recent research into the physiology of GH-transgenesis has revealed cardiorespiratory and metabolic modifications that accompany the increased growth. An elevated routine metabolism has been described for pre-smolt GH-transgenics from ~10 g onwards as well as post-smolt transgenics that also display improvements in oxygen delivery that support the increase in aerobic demand. The onset of cardiorespiratory and metabolic modifications in GH-transgenics has not yet been identified. We therefore investigated the effects of GH-transgenesis and ploidy on metabolic rate, heart rate and ventilatory frequency upon exposure to increasing hypoxia (21–5%) in newly hatched *S. salar* yolk sac alevins. We observed a higher mass-specific metabolic rate in triploid transgenic in comparison to diploid transgenic and non-transgenic as well as triploid non-transgenic conspecifics. While the metabolic rate in all groups decreased with decreasing partial pressure of oxygen (pO_2), the mass-specific metabolic rate of triploid transgenics remained elevated in comparison to all other groups at all pO_2 levels. In contrast, the heart rates and ventilatory frequencies were similar in all groups and remained constant with increasing hypoxia. Our results indicate that the combination of triploidy and GH-transgenesis leads to physiological differences in *S. salar* that occur during early development. The increase in mass-specific metabolic rate observed in triploid transgenics, however, is not reflected in an increase of ventilatory frequency or heart rate to improve oxygen delivery; hence, the increased metabolic rate must reflect better oxygen extraction capabilities.

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16:20 Saturday 30th June 2012

A11.14

Life at the equator: Coral reef fishes may already be living at the edge of their thermal optima

Jodie L. Rummer (ARC Centre of Excellence for Coral Reef Studies, James Cook University, Australia), Christine S. Couturier (University of Oslo, Norway), Jonathan A.W. Stecyk (University of Oslo, Norway), Naomi M. Gardiner (School of Marine and Tropical Biology, James Cook University, Australia), Göran E. Nilsson (University of Oslo, Norway) and Philip L. Munday (ARC Centre of Excellence for Coral Reef Studies, James Cook University, Australia)

Climate change models predict that tropical ocean temperatures will increase by up to 3°C this century. Most impacted might be low-latitude populations because organisms are adapted to a narrower range of temperatures in their local environment. In this study, we investigated temperature-induced differences in metabolic performance between the equatorial populations of six sympatric coral reef fish species.

We acclimated four species of damselfishes and two species of cardinalfishes to temperatures ranging from 29ºC to 34ºC to incorporate their likely lifetime thermal niche (29–31°C, Papua New Guinea, 2º35.765'S; 150°46.193'E) as well as the end of century 3ºC increase predicted by climate change models. Resting and maximum oxygen consumption rates (M_{O2Rest} and M_{O2Max}) were measured for each species at each temperature and used to calculate aerobic scope.

As expected, the aerobic scope declined with increasing temperatures; however, one of the six species investigated, *Chromis atripectoralis*, appears to already be living above its thermal optimum (29°C). The other five species might be at or nearing their thermal optima (approximately 31ºC). Indeed, low-latitude reef fish populations might be more sensitive to ocean warming than higher-latitude populations.

Minor temperature increases (2–3°C) could result in population declines and potentially redistribution of equatorial species.

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16:35 Saturday 30th June 2012

A11.15

Metabolic ecology and links with heart design and climte change in coral reef fishes

Timothy D. Clark (Australian Institute of Marine Science, Australia)

The extent of an animal's home range should be reflected in the capacity of its oxygen transport system, whereby animals with large home ranges should be more athletic. This idea was tested on a range of commercially- and recreationally-important coral reef fishes at the Lizard Island Research Station, Great Barrier Reef, Australia. Species ranged from athletic (e.g. thicklip trevally, *Carangoides orthogrammus*) to sedentary (e.g., common coral trout, *Plectropomus leopardus*), and measurements were made of minimum and maximum oxygen consumption rates ($M_{O2\ min}$ and $M_{O2\ max}$, respectively), ventricle mass, and linear dimensions of the ventricle.

$M_{O2\ min}$ and $M_{O2\ max}$ (28ºC) as well as ventricle mass scaled close to isometrically with body mass (range 86–4,457 g), although there were clear interspecific differences in the intercept of the regressions. All linear dimensions of the ventricle scaled with exponents of 0.31–0.37. In general, $M_{O2\ min}$ and $M_{O2\ max}$ correlated well with estimated home ranges. Relative ventricle mass was also correlated with home range and metabolic performance, but the shape of the ventricle was equally informative (ranging from pyramidal to cranial-caudally elongated).

P. leopardus was selected for further experiments to examine the influence of thermal acclimation on $M_{O2\ min}$ and $M_{O2\ max}$ in the context of a warming climate. $M_{O2\ min}$ increased with temperature (after about one week at 33ºC) and remained elevated after two months, yet there was no discernible change in $M_{O2\ max}$ compared with initial measurements at 28ºC.

These findings suggest a decrease in aerobic scope and possible implications for the metabolic ecology of reef fishes in a warming climate.

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16:50 Saturday 30th June 2012

A11.16

The synergistic effects of high temperature and CO_2 on whole-animal and mitochondrial metabolism of a tropical coral reef fish

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This century, tropical ocean temperatures are predicted to rise by between 1.5 and 3.0°C, while oceans are predicted to decrease in pH by 0.3 to 0.4 units as a result of increasing CO_2. Climate change is suggested to have the greatest impact on tropical marine species, as many species are already living close to their thermal metabolic limits.

Juvenile cinnamon clownfish, *Amphiprion melanopus*, exposed to increased temperature and CO_2 for 31 days have an increased energetic demand, e.g. higher routine metabolic rate (RMR), lower body weight and decreased survival rate. Juveniles hatched from parents that were held in the same experimental conditions, however, grew larger and displayed a lower RMR. Thus, a strong parental effect might be enabling this species to adapt to near-future climate scenarios.

There is a clear energetic component to transgenerational adaptation under these conditions, given the differences in RMR and body size. Our objective therefore was to elucidate the effects of elevated temperature and CO_2 on the metabolism of *A. melanopus*. We investigated haematological parameters and mitochondrial function in relation to whole-animal performance in fish that were held for 18 months in one of nine climate change-relevant treatments: control ambient temperature (28.5ºC) and CO_2 (410 µatm), and cross-factored combinations of moderate (30°C) and high (31.5ºC) temperatures and moderate (610 µatm) and high (950 µatm) CO_2. Routine and maximal O_2 consumption rates were determined and aerobic scope calculated for adult male fish. Gill and muscle mitochondrial respiration and enzyme profiles were measured to understand metabolic capacity.

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17:05 Saturday 30th June 2012

A11.17

Limpets, hanging on in a warming world

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To test their relative vulnerability to different scales of climate warming, the thermal reaction norms of four intertidal nacellid limpets from Antarctica (*Nacella concinna*), New Zealand (*Cellana ornata*), Australia (*C. tramoserica*) and Singapore (*C. radiata*), were compared across environments with different temperature magnitude and variability. Lethal limits were measured alongside 'duration tenacity', which measures the thermal reaction norm of limpet muscular fatigue as they clamp to the substratum and resist a pulling force.

Duration tenacity did not follow a typical aerobic scope curve but was best described by linear fits, with either a single break point at an optimum temperature (*N. concinna*, *C. ornata*, *C. radiata*), or two break points with an optimum temperature range (*C. tramoserica*). The optimum temperature for tenacity increased in warmer environments, from 1.0°C (*N. concinna*) to 14.3ºC (*C. ornata*) to an average of 18.0°C (*C. tramoserica*) to 27.6ºC (*C. radiata*). The temperature limits for duration tenacity were most consistently correlated with mean maximum air temperature, maximum sea surface temperature and lowest summer maximum logger temperature. The upper temperature limits of tropical *C. radiata* had a lower thermal buffer, with respect to mean environmental temperature, than temperate species from New Zealand and Australia. The two temperate species, however, had a lower thermal buffer with respect to extreme air temperatures, which are unpredictable at both these locations.

Intertidal animals live at the highly variable interface between terrestrial and marine biomes and their vulnerability to changing climate will depend on the scale and the predictability of any changes.

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17:20 Saturday 30th June 2012

A11.18

Wild geese do not increase flight behaviour prior to migration

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Hypertrophy of the flight muscles is regularly observed in birds prior to long-distance migrations. We tested the hypothesis that a large migratory bird would increase flight behaviour prior to migration in order to cause hypertrophy of the flight muscles and up-regulate key components of the aerobic metabolic pathways.

Implantable data loggers were used to record year-round heart rate in six wild barnacle geese (*Branta leucopsis*), and the amount of time spent in flight each day was identified. Time in flight per day did not significantly increase prior to either the spring or the autumn migration, both between time periods prior to migration (five, 10 and 15 days), or when compared to a control period of low activity during winter.

The lack of a significant increase in flight prior to migration suggests that approximately 22 minutes per day is sufficient to keep the flight muscles in condition for prolonged long-distance flight. This apparent lack of a requirement for increased flight activity prior to migration might be attributable to pre-migratory mass gains in the geese, increasing the workload during short flights, potentially prompting hypertrophy of the flight muscles.

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Poster Session – Saturday 30th June 2012

A11.19

Integrating -omics with organisms: The toolbox for testing metabolic responses of larval echinoderms to rearing in elevated CO_2 seawater

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The rapid acidification of our oceans through equilibration with anthropogenically-produced CO_2 is one of the greatest ecological issues of our time. A growing body of research suggests that levels capable of altering organismal, biochemical and genetic processes in water-breathing animals will be reached by the end of this century. Unfortunately, the ecophysiologist's toolbox is still desperately wanting for sensitive techniques able to help elucidate the physiological mechanisms responsible for these alterations.

In this research, we have used a well-described larval echinoderm model and cutting-edge technologies, such as high-resolution respirometry and metabolomic profiling, to develop novel protocols for assessing effects of acidification of the oceans on metabolically-relevant parameters. These parameters include metabolic rate and scope, mitochondrial function, metabolite profiles, and protein and gene expression.

Our data suggest that rearing in seawater equilibrated with near-future CO_2 levels alters mitochondrial function, providing a mechanism to explain our observations of developmental delay and malformation, and alterations in the whole-animal rate of oxygen consumption. We will integrate these findings with metabolomic, transcriptomic and proteomic profiles developed through our own and others' research. Overall, this research project aims to create tools to better determine the physiological impacts of ocean acidification on echinoderm larvae, and ultimately elucidate the potential physiological challenges faced by marine ecosystems in the near future.

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Poster Session – Saturday 30th June 2012

A11.20

Compensatory strategies in goldfish and common carp in response to water-borne ammonia

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High environmental ammonia (HEA) is becoming a major constraint in aquatic habitats. This can result in major issues, which eventually lead to acute as well as chronic effects varying between species. As a consequence, the physio-biochemical processes are disturbed. Recent studies have revealed a new insight in ammonia excretion at the gills, involving ammonia transporters/channels and most prominently rhesus glycoproteins. The mechanisms behind these transporters and associated processes are still controversial and can vary among different fish species.

The main focus of our work is to investigate various compensatory mechanisms for coping with ammonia in goldfish and common carp, each having its own level of sensitivity to the HEA. Both fish species were exposed to 1 mM of ammonia during a period of zero (control), three and 12 hours and one, two, 3.5 and 7.5 days. Measurements of Na^+/K^+-ATPase and H^+-ATPase activity and gene-expression in the gills followed a distinguished pattern along with the exposure duration and changes were more prominent in goldfish. Consequently, changes in the plasma ion (Na^+, K^+, Cl^-) concentrations were obvious. The decline in energy budget in response to HEA was more distinctive in common carp. Plasma ammonia, lactate and urea concentrations were analysed as well. In brief, HEA affected the metabolic, physiological and ion-regulatory responses in both species.

Our results show that goldfish have better strategies to cope with ammonia toxicity, thus explaining their higher resistivity against HEA compared to common carp. Expression kinetics of rhesus glycoprotein genes in these species will be conducted in our future work.

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Poster Session – Saturday 30th June 2012

A11.21

Transcriptional origins of variation in cytochrome c oxidase activity in fish

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In many fish species, cold exposure triggers an increase in mitochondrial oxidative capacity, indicated by cytochrome c oxidase (COX) activity. Central to the control of mitochondrial biogenesis in mammals is the transcriptional co-activator PGC-1α (peroxisome proliferator-activated receptor-γ co-activator-1α). It co-activates a number of DNA-binding proteins that have been identified as regulators of mitochondrial genes: nuclear respiratory factors (NRF) 1 and 2, host cell factor 1 (HCF1), retinoid X receptor α (RXRα), estrogen related receptor α (ERRα), thyroid hormone receptor α-1 (TRα-1), and peroxisome proliferator-activated receptors (PPARs).

Interestingly, previous studies suggest that PGC-1α does not play the same role in fish. The importance of the complex network of DNA-binding proteins, however, has yet to be evaluated in fish models. We explored the role of this pathway in response to cold acclimation in various tissues in goldfish (*Carassius auratus*), measuring COX activity, the mRNA and protein levels of some key regulators.

COX activity was only elevated in cold fish compared to warm in the white and red muscles and the gills. In white, red and cardiac muscle, PGC-1β mRNA increased, whereas PGC-1α mRNA did not. Increases in mRNA were also seen for NRFs, HCF1, ERRα, PPARβ/δ and RXRα. Despite this general increase in transcription factor mRNA levels, nuclear protein levels only increased for NRF-1.

Collectively, our data show that the control of mitochondrial proliferation in fish seems to be quite different from mammals with respect to many key regulators, yet, the role of NRF-1 and PGC-1β in this network is becoming more certain.

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Poster Session – Saturday 30th June 2012

A11.22

Salmonids respond differently to high external ammonia than Cyprinids: a comparative study on the regulation of Na^+ flux, ammonia transporters and associated mechanisms

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The mechanisms for ammonia excretion at the gill epithelium in freshwater fish have not yet been fully revealed. Current hypotheses include the diffusion of NH_3, most likely facilitated by rhesus proteins, followed by trapping as NH_4^+ in the acidified gill boundary layer as well as interactions between the transport of Na^+ and NH_4^+ through Na^+/NH_4^+ exchangers and the substitution of K^+ by NH_4^+ in basolateral Na^+/K^+-ATPase transport. The direct interplay of ammonia and sodium transport, however, has not been unravelled. In the present study we therefore examined the compensatory response of three commercially-important freshwater fish: a sensitive salmonid, the rainbow trout *Oncorhynchus mykiss*, and less sensitive cyprinid species such as common carp, *Cyprinus carpio* and goldfish, *Carassius auratus* when exposed acutely (three hours) and chronically

(up to one week) to high environmental ammonia (1 mM).

Effects on plasma ammonia and urea levels, gill sodium transport and other markers of gill permeability such as net K^+ flux, ammonia/urea flux, diffusive water flux (by $3H_2O$ exchange) and transepithelium potential, were assessed. The alteration in sodium flux and ammonia excretion was preceded by the change in gill activity and mRNA transcript of Na^+/K^+-ATPase and H^+-ATPase. The mRNA expression of rhesus proteins (RhCg1a, RhCg1b and Rhbg) and the urea transporter were differentially regulated in three species.

Overall, our study explains why the trout is more disturbed by ammonia exposure and shows a weaker compensatory response in most parameters compared to the carp or the goldfish. Moreover, the responses among carp and goldfish were not considerably different.

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Poster Session – Saturday 30th June 2012

A11.23
Thermal habitat and tolerance of triploid rainbow trout

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In sport fishing lakes with depleted fish stocks, sterile triploid rainbow trout are stocked to prevent hybridization with wild stocks and satisfy preference for the appearance of triploids. Unfortunately, triploid trout lake survival and growth are usually suboptimal, possibly due to poor temperature tolerance. Here we investigated thermal habitat and temperature tolerance of triploids.

Over a six-month period in the lakes, fish spent large portions of each day near the surface despite cooler, oxygenated water being available. In one of these lakes, when summer temperatures peaked, both ploidies spent most of their time close to 20ºC. Laboratory temperature-tolerance experiments suggest that 20ºC is well above their optimal temperatures (where trout have the broadest aerobic scope) and approaches maximum acute thermal tolerance.

We have been investigating several aspects of cardiorespiratory physiology related to reduced triploid temperature tolerance and discovered that both ploidies have similar optimal and cardiac arrhythmia onset temperatures and *in vitro* blood O_2 loading at high temperatures. Triploids have a lower heart rate than diploids across a range of temperatures, however, suggesting a different cardiac strategy to deliver oxygen during warming. Lower heart rates could reflect lower cardiac output or greater reliance on stroke volume, but how this difference relates to fish survival at high temperature requires further work. Since rainbow trout preferred to inhabit supraoptimal temperatures in the lake studied and given the small temperature window for survival, even small increases in temperature with climate change are likely to have important implications to stocking lakes with triploid rainbow trout.

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Poster Session – Saturday 30th June 2012

A11.24
Subcellular differences in handling excess copper in three freshwater fish species contributes greatly to their differences in sensitivity to copper

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Changes in subcellular metal distribution can provide insights in metal toxicity and tolerance. We exposed rainbow trout, common carp and gibel carp to 50 µg/l copper (Cu) for up to one month. These species are known to differ in their sensitivity to Cu, with gibel carp being the most tolerant and trout the most sensitive.

Although the liver of rainbow trout showed extremely high Cu levels, the amount of Cu accumulated in gills was lower compared to common and gibel carp. At the subcellular level, rainbow trout gills distributed the additional Cu exclusively to the biologically-active metal (BAM) pool (heat denaturable fraction and organelle fraction). A similar response was seen in the gill tissue of common carp, although the percentage of Cu in the BAM pool was lower compared to trout. Gill of gibel carp accumulated more Cu in the biologically-inactive metal (BIM) pool (heat-stable fraction and metal-rich granule fraction). The liver of rainbow trout seemed much more adequate in handling the excess Cu compared to its gills: storage of Cu in the BIM pool increased. The high percentage of Cu in the metal-rich granule and heat-stable fraction in liver of common carp, and especially gibel carp with the better Cu handling in gill tissue, pointed highlighted the ability of carp species to minimize the disadvantages related to Cu stress.

The differences in Cu distribution at the subcellular level strongly reflect fishes' capacity to handle excess Cu, being one of the greatest contributors to their difference in sensitivity to Cu.

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Poster Session – Saturday 30th June 2012

A11.25
Experimental increase in pectoral muscle size leads to an improved cold tolerance in free-living Black-capped chickadees wintering in Quebec (Canada)

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In winter, resident bird species living at northern latitudes exhibit changes in metabolic performance in response to cold weather conditions. Basal metabolic rate (reflecting minimal maintenance energy costs) and maximal thermogenic capacity (M_{sum}, a measure of cold tolerance) are higher in winter relative to other seasons. Birds undergoing cold stress produce heat by shivering, so it is generally assumed that M_{sum} depends on pectoral muscle size, something that has been supported by some correlation studies. This relationship has, however, yet to be demonstrated experimentally.

To investigate the relationship between muscle size and cold endurance, we manipulated pectoral muscle mass in free-living Black-capped chickadees (*Poecile atricapillus*). We clipped half of the flight feathers in experimental individuals and compared them to control birds with intact wing surface area captured at the same time. Results show that, compared with controls (n=15), 'clipped' chickadees (n=9) had similar body mass, haematocrit level, amount of body fat and basal metabolic rate, but had larger pectoral muscles (+17%) and reached a higher M_{sum} (+9%). These findings highlight the fact that, independently of weather or body condition, having larger muscles leads to improved cold tolerance.

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Poster Session – Saturday 30th June 2012

A11.26
Effects of ocean acidification on five major marine animal taxa: A synthesis

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In parallel to driving the on-going trend of climate warming, anthropogenic CO_2 penetrates into marine surface waters causing ocean acidification. Since being recognized as a marine stressor in 2004, anthropogenic ocean acidification has repeatedly been suggested as a major future challenge for marine organisms and ecosystems. Here we analyse the effect of various species-specific responses of corals, echinoderms, molluscs, crustaceans and fish at the physiological, behavioural and molecular levels, including a variety of life stages. To gain a comprehensive picture in these major taxa, sensitivities to several ranges of sea water pCO_2 (500–650, 651–850, 851–1,370, 1,371–2,900, 2,901–10,000 and >10,000 µatm) were evaluated.

The assessment identifies a high proportion of negatively-affected coral, echinoderm, mollusc and fish species, even at the lowest CO_2 treatments; whereas crustaceans were less sensitive. Only a few species of these important players in marine food webs have been studied at the lowest pCO_2, however, and our knowledge of responses to near-future changes (until 2050) is rudimentary. Furthermore, the effects of combined stressors need to be investigated to yield reliable predictions of the impacts of climate change.

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Poster Session – Saturday 30th June 2012

A11.27
Physiological impacts of temperature increase in ice krill (*Euphausia crystallorophias*): HSP70s responses

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Polar regions are the first to suffer the effects of global warming. An elevation of 1°C in the temperature of seawater has been observed in the peninsular region of Antarctica. In this context, estimation of the physiological impact of a potential increase in temperature on species located in these areas is a priority in terms of biodiversity management.

Euphausiids (krill) are a keystone species in the Antarctic trophic chain and feed zooplankton as well as large carnivorous mammals and birds of the region. The physiological response of krill species (*Euphausia superba and E. crystallorophias*) to an increase in ocean temperature could be a pertinent marker to predict the evolution of Antarctic animal communities.

An approach combining laboratory experiment and transcriptomic has been implemented to study the molecular response of ice krill during heat stress. Heat shock protein (HSP) has been chosen as the molecular marker for their chaperoning role in thermal stress response. High-throughput sequencing (Illumina) allowed us to characterize several HSP families including several isoforms in the HSP70 family. The expression levels of five HSP70 isoforms were studied in response to an artificial heat stress at 3ºC and 6ºC using quantitative polymerase chain reaction. This work will discuss the preliminary results obtained by real-time quantitative polymerase chain reaction expression analysis assay for HSP70 genes in *E. crystallorophias*.

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Poster Session – Saturday 30th June 2012

A11.28
Are mitochondria at the heart of temperature induced cardiac failure? A study of closely related fish species from different thermal habitats

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Predictions of climate change-mediated rises in ocean temperatures suggest that ectothermic hearts might place tight constraints on many marine species. For many aquatic species, the upper temperature limit (T_{max}) and the heart failure (HF) temperature (T_{HF}) is only a few degrees away from their current environmental temperatures (Portner, HO and Knust, R. *Science*, 2007; 315: 95–97). The window between T_{max} and T_{HF} appears to be narrower in tropical than for temperate fishes, and only slight temperature increases appear to induce HF. Why heat-stressed hearts fail remains unresolved.

In fish, HF due to elevated temperatures can result from energy and/or oxygen supply disruptions to and from mitochondria in cardiac cells (Somero GN, *Integ. Comp. Biol.* 2002; 42: 780–789). This study targets mitochondria, as damaged mitochondria could be potential sources of reactive species and might trigger apoptosis or fail to produce enough ATP to sustain a heartbeat.

We are testing the influence of elevated temperatures on heart mitochondria in three closely-related fish wrasse species that occupy cold temperate (*Notolabrus cinctus*), temperate (*Notolabrus celidotus*) and tropical (*Thalassoma lunare*) habitats. Across species, a substantial drop in phosphorylation efficiency (inferred from polymerase chain reaction) occurred below or at $T_{max,}$ suggesting mitochondrial ATP supply might compromise heart function at elevated temperatures. We also found that the apparent K_M for pyruvate rises about 100-fold after T_{max}. These data suggest that mitochondrial integrity plays a role in thermal stress tolerance and may therefore limit species distributions.

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Poster Session – Saturday 30th June 2012

A11.29
Physiological sensitivity along a latitudinal gradient: A study case in marine ectotherms

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Among the many different components of global environmental change, global warming is projected to be the largest human-induced disturbance placed on natural ecosystems. Environmental temperature (T_a) is the abiotic factor with major incidence in the physiology and ecology of ectotherms: any trait (e.g. growth, reproduction and physiology) will change as T_a changes – a relationship described by a thermal performance curve. The amplitude of this curve is best captured by two parameters: the minimum (CT_{min}) and maximum (CT_{max}) critical temperatures.

Global analyses of both CT_{max} and CT_{min} have shown that both traits are affected by latitude, suggesting at least some adaptation to T_a. An important but often neglected factor in these global analyses, however, is the inter-population variation in species distributed across broad geographic (and latitudinal) ranges: if there is local adaptation to $T_{a,}$ then species should be more vulnerable to climate change. We carried out a geographic analysis of five populations of five crustacean species along a latitudinal gradient of almost 3,000 km. We determined CT_{min} and CT_{max} and evaluated the following three questions:

- Is there a latitudinal pattern in both CT_{max} and CT_{min}?
- Is this pattern similar among the different species?
- Is the relationship between both tolerances also affected by latitude?

We found that: CT_{max} shows a latitudinal pattern but not CT_{min}; the latitudinal pattern was species-specific; and the relationship between both tolerances and latitude was also dependent upon the particular species.

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Poster Session – Saturday 30th June 2012

A11.30
Interactions between chemical exposure and heat stress in the flounder (*Platichthys flesus*)

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Human activities since the beginning of the last century have been responsible to the increase in ocean temperature and the accumulation of polluting products in marine systems. We studied the interaction between these two stresses on the flounder (*Platichthys flesus*) by measuring the oxygen consumption of cardiac tissues.

Four-hundred fishes were divided into four groups: two uncontaminated groups at 9ºC (control) and 24ºC, and two contaminated groups at 9ºC and 24ºC. Cardiac ventricles were sampled 10 and 40 days after contamination. For each sampling, oxygen measurements on were performed permeabilized fibres. Maximal oxygen consumption rates ($M_{O2\ Max}$ in pyruvate malate and ADP) and oxygen consumptions in the presence of oligomycin were measured. These two measures allowed the proton leak to be calculated. As expected, at the end of the temperature rise (at day 10), the $M_{O2\ Max}$ was significantly increased independently of contamination. At day 40, the fishes were acclimated to hyperthermia: no difference of $M_{O2\ Max}$ was observed between the 9°C and 24ºC groups. Proton leaks were also altered by hyperthermia: at day 10, both contaminated and control groups exhibited decreased H^+ leaks. Interestingly, at day 40 this difference no longer existed in control fishes; while in contaminated fishes the H^+ leak was still different from the reference value at 9°C.

In conclusion, the data demonstrated an effect of temperature on the oxygen consumption of cardiac tissue. In addition, they indicated the existence of an interaction between contamination and temperature: contamination appeared to reduce the ability of flounders to adapt to hyperthermia.

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Poster Session – Saturday 30th June 2012

A11.31
Aerobic performance of Atlantic salmon in a warmer future: No difference between a north Norwegian and a French population

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The North Atlantic Ocean can expect rising water temperatures by the end of the century, and the potential effect of this on the performance of wild Atlantic salmon (*Salmo salar*) is not presently understood. The difference between resting and maximum oxygen consumption – the aerobic scope – determines how much energy fish can invest into feeding, growth and reproduction. Resting and maximum oxygen consumption rise with temperature at different rates, and beyond the optimal temperature a further increase in temperature will reduce the aerobic scope. Thus, long before water temperature reaches the critical temperature that is immediately lethal it will reduce the energy available for feeding, growth and reproduction.

A reduced aerobic scope has now been identified as a key factor threatening the population survival of fish due to ocean warming. We have compared the aerobic scope of two populations more than 3,000 km apart – one from northern Norway (Alta: fish experiencing average river temperatures between 0 and 15ºC) and the other from France (Dordogne: fish experiencing average river temperatures between 5 and 25ºC). Fry were raised at 12, 14, 16, 18 and 20ºC for a month after their first feeding and their aerobic scope was determined at all temperatures. For all acclimation groups, resting and maximum oxygen consumption and aerobic scope increased with temperature, but surprisingly there were no significant differences between the two populations, indicating a lack of local temperature adaptation and the ability for both populations to cope with high temperature (around 20ºC).

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Poster Session – Saturday 30th June 2012

A11.32
Elevated CO_2 enhances the aerobic scope of a coral reef fish

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The uptake of anthropogenic CO_2 by the ocean has been suggested to have an impact on marine ecosystems by decreasing the respiratory capacity of fish and other water-breathers. We investigated the aerobic scope of the spiny damselfish, *Acanthochromis polyacanthus*, from the Great Barrier Reef, Australia, when exposed to CO_2 conditions predicted for the end of the century (946 µatm CO_2).

Surprisingly, resting O_2 consumption rates were significantly lower and maximum O_2 consumption rates were significantly higher in high-CO_2 exposed fish compared to control fish (451 µatm CO_2). Consequently, high-CO_2 fish exhibited an unexpected increase in absolute (38%) and factorial (47%) aerobic scopes. Haematological and muscle water changes associated with exercise were unrelated to CO_2 treatment, with one exception: high-CO_2 fish significantly decreased haematocrit from 78.7 to 67.6% post-exercise, suggesting either increased water uptake to compensate for the elevated ion influx over an enlarged respiratory surface area (as indicated by the increased O_2 uptake) or blood flow redistribution during exercise.

Thus, contrary to predictions, our results suggest that elevated CO_2 might enhance the aerobic scope of some fish species. Understanding the variability among species regarding the effects of CO_2 on aerobic scope will be critical in predicting the impacts of ocean acidification on marine communities and ecosystems.

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Poster Session – Saturday 30th June 2012

A11.33
Morpho-functional traits associated with heat and hypoxia tolerance in fish

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Prevailing biotic and abiotic conditions set the energetic cost of adaptation to a given habitat, with repercussions on indices of fitness such as growth. In fish, growth presents a marked inter-individual variability, suggesting the existence of an underlying functional trade-off. In order to elucidate the characteristics of this trade off, and in particular whether it involves environmental adaptation abilities, we examined and compared the morphofunctional characteristics of two strains of rainbow trout (a fast growing and a slow growing strain).

Experimental data showed that fast growers were more hypoxia-tolerant than slow growers. The fast-growing strain was found to be less tolerant to heat than the slow-growing one. In both strains, body mass

was found to be a strong determinant of hypoxia and hyperthermia tolerance, but large inter-individual variation still persisted within each strain. Analysis of residues highlighted that whereas none of the individual morphometric traits (mass of liver, gill, heart ventricle and digestive tract), energetic traits (AMR, SMR and permeabilized cardiomyocytes maximal oxygen consumption) correlated with hypoxia tolerance. Permeabilized cardiomyocytes maximal oxygen consumption correlated well with individual tolerance to heat. Moreover, the mass exponents of liver, gill, heart ventricle and digestive tract were found larger than 1, ranging between 1.18 and 1.23.

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Poster Session – Saturday 30th June 2012

A11.34

Thermal effects on the metabolic performance of a tropical fish, the barramundi (*Lates calcarifer*): Implications for climate change and the concept of oxygen-limited thermal tolerance

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

Global warming is causing water temperatures in some areas to rise at an unprecedented rate, leading to decreased abundances and distribution shifts of many fish species. The physiological driver for the changes in population structure has been proposed to be a mismatch between oxygen supply and demand at high temperatures when the capacity of the ventilatory and circulatory systems to deliver oxygen to the respiring tissues is exceeded (i.e. oxygen-limited thermal tolerance), causing a decrease in aerobic scope (maximum minus minimum oxygen consumption rate). To test this, we measured oxygen consumption rates of barramundi (*Lates calcarifer*) at 23, 29, 35 and 38ºC.

Although ~30ºC is known to be the optimal temperature (T_{opt}) for a range of important life history traits in this species, we found that the aerobic scope continued to increase with temperature (5.9 mgO_2/min/kg at 23ºC to 12.5 mgO_2/min/kg at 38ºC) until the animal succumbed abruptly with a further increase in temperature to 41ºC. The finding of a continual increase in aerobic scope up to 38ºC (a temperature that is unlikely to be encountered at any point in the lifecycle of the species) contrasts with the assumption that the preferred temperature of the species should correlate with T_{opt} for aerobic scope, and it suggests that thermal constraint does not lie with the ventilatory and circulatory systems in barramundi.

Variation in the intraspecific metabolic performance of barramundi will also be discussed with the aim of highlighting the importance of individual traits in determining overall resilience to a changing environment.

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Poster Session – Saturday 30th June 2012

A11.35

Maximum intrinsic heart rate and oxygen uptake of tropical reef fishes at elevated temperature: compensatory changes after warm acclimation

Jonathan A.W. Stecyk (University of Oslo, Norway), Christine S Couturier (University of Oslo, Norway), Jodie L. Rummer (James Cook University, Australia), Philip L. Munday (James Cook University, Australia) and Göran E. Nilsson (University of Oslo, Norway)

The aerobic scope of tropical reef fishes decreases drastically at temperatures merely 2–3°C higher than they presently experience (~29°C) but the underlying physiological mechanisms and whether compensatory thermal acclimation can occur remains unclear. To examine the contribution of cardiac limitation at high temperature, we measured how the maximum intrinsic heart rate (f_H) of a number of tropical reef fish (representing damselfishes, cardinalfishes and grunters) acclimated to ~29ºC or 32ºC for eight to 10 days was affected by acute warming from 29ºC up to 34ºC. Resting and maximum oxygen consumption (M^o and aerobic scope were determined for two species across the temperature range to examine the relationship between f_H and aerobic performance.

For 29ºC-acclimated fishes, f_H increased with Q10 values near 2 during acute warming from 29ºC to 32ºC. The f_H plateaued or decreased above 32ºC in all species. Acclimation to 32ºC resulted in the resetting of f_H to a lower frequency such that no decrease or plateauing of f_H occurred with increased temperature. A similar pattern of change was observed for degrees of high-temperature-acclimated fish.

Thus, brief acclimation to slightly elevated temperature resulted in compensatory changes in maximum cardiac activity and degrees.

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Poster Session – Saturday 30th June 2012

A11.36

From winter to summer in two weeks: Acute thermal tolerance in the freshwater painter's mussel (*Unio pictorum*)

Glenn J. Lurman (University of Bern, Switzerland), Tess Hoppeler (University of Bern, Switzerland) and Hans H. Hoppeler (University of Bern, Switzerland)

Lake Murten, on the Swiss plateau, experiences large variations in water temperature, from 0–1°C in winter to 27ºC in summer. During the 'spring thaw', the temperature increases by 0.2ºC per day over several months. Water temperature also varies considerably over shorter time spans, by up to 8ºC within a few days, equating to a rate of 1–2ºC per day. Given the stress this variation must exert on the lake fauna, we wanted to examine the effect of an acute thermal manipulation on the endemic painter's mussel (*Unio pictorum*). Winter acclimatized (4–5°C) U. pictorum were exposed to temperatures ranging from 0.5°C to 36°C with increments of 2°C per day. Oxygen consumption (M_{O2}) and heart rate (HR) were 0.007 ±0.001 mg O_2 h^{-1} and 3.14 ±0.19 bpm, respectively, at the acclimatization temperature of 4°C. Initial cooling to 0.5°C caused M_{O2} to increase to 0.017 ±0.002 mg O_2 h^{-1} and heart rate decreased slightly to 2.11 ±0.25 bpm. Subsequent heating to 32°C increased M_{O2} to 0.592 ±0.115mg O_2 h^{-1} and heart rate to 23.2 ±3.67 bpm. The M_{O2} plateaued between 28 and 32°C and then fell to 0.153 ±0.066 mg O_2 h^{-1} at 34°C while heart rate plateaued at 28°C and remained high at 34°C. An Arrhenius break in M_{O2} was observed at 22°C; however the upper critical temperature for *U. pictorum* appears to be 32°C.

Despite the high critical temperature, questions remain about the ability of these mussels withstand sustained high temperatures, especially given how close they come to their upper critical temperature in summer.

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Poster Session – Saturday 30th June 2012

A11.37

The effects of elevated temperature and acidity on marine calcified and non-calcified invertebrates in winter

Yaser Saad Binnaser (Newcastle University, UK), John C. Bythell (Newcastle University, UK) and Richard M. Bevan (Newcastle University, UK)

Anthropogenic activities have elevated the concentration of CO_2 in the atmosphere. This increased CO_2 has caused a rise in the surface

temperature of the oceans and an increase in the levels of ocean acidity. We studied the effect of changing temperature and acidity levels by subjecting marine invertebrates (two calcified and two non-calcified species) to the climatic conditions predicted in winters of 2050 and 2100.

Animals were given two weeks to adapt to the climatic conditions and then monitored for a further six weeks during which growth and metabolic rates were measured. The responses of the animals to the predicted changes varied between species. There was an increase in mortality in *Ascidiella aspersa* and in *Mytilus edulis* under 2050 and 2100 conditions; whereas *Littorina littorea* and Actinia equina had high survivorship. Growth rates were reduced in *A. aspersa* under both 2050 and 2100 conditions, but increased in both *A. equina* and *L. littorea* under the same conditions. The growth rates of *M. edulis* decreased under 2050 conditions but increased under 2100 levels. Metabolic rates were elevated under conditions of 2050 and 2100 in *M. edulis*, *L. littorea* and *A. equina*. This increase in metabolic rate is most likely due to the direct link between temperature and metabolism and, provided there are sufficient resources present, most animals would appear to respond positively to the changes in conditions. Importantly, even though these experiments were undertaken in winter temperature conditions, under these relatively small temperature changes *A. aspersa* was adversely affected.

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Poster Session – Saturday 30th June 2012

A11.38
Torpor flexibility is mainly constrained by body size

Pauline Vuarin (UMR 7179 CNRS, Muséum National d'Histoire Naturelle, France), Melanie Dammhahn (German Primate Centre, Germany) and Pierre-Yves Henry (UMR 7179 CNRS, Muséum National d'Histoire Naturelle, France)

When facing environmental variations, some animals can compensate for the new constraints by adjusting their behaviour and physiology. Hibernation and daily torpor (heterothermy) are efficient physiological responses to overcome energetic constrains based on saving energy. Understanding what determines individual flexibility in the use of such strategies is of major interest in climate change biology.

In Madagascar, during the dry season animals face food and water shortages combined with low ambient temperature. The grey mouse lemur (*Microcebus murinus*), a small primate, reduces its energy expenditure by exhibiting daily torpor when conditions become unfavourable. During the onset of the dry season, we monitored the skin temperature of free-ranging individuals in the dry forest of Kirindy (west of Madagascar).

We show that minimal skin temperature depends on ambient temperature, but also on body size and body condition. At a given ambient temperature, larger individuals and/or those in better body condition exhibited deeper torpor than smaller sized ones. The lightest individuals remained normothermic however, suggesting that fattening before the dry season determines an individual's ability to use torpor. At the individual level, reaction norms of skin temperature to ambient temperature (i.e. torpor flexibility) depended on body size, but not on body condition. This dependence is expected if larger individuals achieve higher energy reserves because they have proportionally more fat per unit of body mass or if they are dominant and have better access to limited resources. Thus, body size appears to be a major determinant of the ability to rely on torpor-based energy-saving to overcome energetic challenges.

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Poster Session – Saturday 30th June 2012

A11.39
Stress-marker protein expression in the freshwater painter's mussel (*Unio pictorum*) during an acute temperature increase

Glenn J. Lurman (University of Bern, Switzerland), Alexander P. Atanassoff (University of Bern, Switzerland) and Annette Draeger (University of Bern, Switzerland)

Changes in temperature can have significant impacts upon plasma membrane structure and cell function within the organs of ectotherms. Lake Murten, on the Swiss plateau, experiences considerable thermal variation on long-term seasonal and short-term time scales. We have examined the effect of an acute temperature increase on the degree in protein expression of cellular stress-markers, such as heat-shock proteins (HSP) 90 and 70 and members of the annexin protein family in the endemic painter's mussel (*Unio pictorum*).

Exposure to temperatures ranging from 0.5ºC to 36ºC with increments of 2ºC per day, revealed significant increases in the expression of HSP90 and 70, and annexin A6 and 5. Furthermore, these stress markers display tissue-specific up-regulation and distribution. HSP expression was up-regulated in all three tissues sampled – namely gill, foot and mantle – with the greatest response seen in the gill. All tissues showed significant up-regulation of annexin A6, with the greatest up-regulation in the gill. Annexin 5 was only up-regulated in the foot, and to a lesser degree in the mantle. Thus, the up-regulation of these stress-markers and their distribution points to a differential, organ-dependent reaction to an increase in temperature.

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Poster Session – Saturday 30th June 2012

A11.40
Ocean acidification may alter behaviours in a temperate fish

Fredrik Jutfelt (University of Gothenburg, Sweden), Sam Dupont (University of Gothenburg, Sweden), Trond Amundsen (Norwegian University of Science and Technology, Norway), Roman Motyka (University of Gothenburg, Sweden) and Elisabet Forsgren (Norwegian Institute for Nature Research, Norway)

The world's oceans are becoming increasingly acidified due to anthropogenic CO_2 emissions. This could have large effects on marine ecosystems, and has been described as a major threat to marine life. How marine fishes are affected is largely unclear. Altered sensory responses and behaviour have been found in coral reef fishes, including increased boldness and activity, and altered responses to olfactory and auditory cues.

As research has focused on tropical reef fish, it has been unclear how widespread the behavioural effects are. Here we show the effects of high CO_2 levels on embryonic development and larval behaviour and in a temperate marine goby (*Gobiusculus flavescens*).

We followed natural reproductive activities (courtship, mating, spawning, embryonic and larval development) in aquaria with elevated CO_2 (1,300 µatm CO_2) or control seawater (400 µatm CO_2). Interestingly, increased CO_2 led to a strong increase in larval phototactic response (swimming towards a light source) and hyperactivity. Increased CO_2 also resulted in higher egg mortality and developmental malformations among embryos. As in larval fish, juveniles acclimated to 1,200 ppm CO_2 were hyperactive compared to control fish.

This study shows that abnormal behaviour caused by high pCO_2 can be widespread in marine fish in terms of geography, species and life stages.

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Poster Session – Saturday 30th June 2012

A11.41

Temporal dynamics of acclimation to elevated temperature on metabolic scope and specific dynamic action in shorthorn sculpin (*Myoxocephalus scorpius*)

Henrik Seth (University of Gothenburg, Sweden), Michael Axelsson (University of Gothenburg, Sweden), Albin Gräns (University of Gothenburg, Sweden) and Erik Sandblom (University of Gothenburg, Sweden)

With the present ocean warming, it is becoming increasingly important to determine the metabolic consequences of temperature increase in fish. Several studies have addressed this question, but most have involved acute (hours to days) temperature changes. Although such experimental protocols can provide useful physiological information, they clearly fail to determine the capacity to acclimate physiologically during long-term thermal challenges: i.e. climate change.

Here, the long-term effects of thermal acclimation on aerobic scope and specific dynamic action (SDA), two physiological traits with profound impact on fitness, were investigated in the shorthorn sculpin (Myoxocephalus scorpius). Fish were acclimated to 10ºC for several months. A control group was then kept at 10ºC, while two other groups were transferred to 16ºC, which is close to the upper temperature limit for this species. Metabolic scope was measured weekly at the two temperatures for nine weeks, while SDA was measured every other week in the second 16ºC group.

Our results show that there is a large increase in basal metabolism upon transfer to 16ºC, which reduces metabolic scope. The SDA initially occupies the entire metabolic scope leaving little room for activities beyond digestion. Thermal acclimation, however, resulted in a gradual depression of basal metabolism back to the 10ºC value, which partly restores metabolic scope. The SDA is no longer limited by the scope and as a consequence there is an increased capacity to sustain activities other than just feeding.

In conclusion, the shorthorn sculpin has the capability to long-term acclimate to substantial increases in temperature.

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Poster Session – Saturday 30th June 2012

A12 – LINKING PHYSIOLOGY WITH BEHAVIOUR AND PERSONALITY

A12.1
Tinbergen (almost) 50 years on: Integration in nutritional ecology

David Raubenheimer (Massey University, New Zealand), Stephen J. Simpson (University of Sydney, Australia) and Alice Tait (Massey University, New Zealand)

A hallmark of nutritional ecology is its integrative scope: it addresses questions about the interaction of animals with their environment by drawing on methods and concepts from across the biological sciences. In his famous paper 'On aims and methods in ethology' (1963), Niko Tinbergen made a similar point about his field, ethology. Tinbergen defined ethology as "the biological study of behaviour", and set out to demonstrate the "close affinity between Ethology and the rest of Biology". Tinbergen did so by showing how the study of behaviour related to the four "major problems of Biology" – causation, survival value, evolution and ontogeny. These problems, which are often referred to as "Tinbergen's four questions", have been hugely influential, and almost 50 years later are still considered a benchmark in integrative biology.

In this talk we relate Tinbergen's questions to nutritional ecology. We demonstrate that Tinbergen's 'four questions' scheme needs to be modified and expanded to encompass modern nutritional ecology, and present a modelling framework – the geometric framework for nutrition – which serves as an integrator for the expanded set of fundamental questions. We illustrate using examples from our own research on nutrition-related behaviour and physiology.

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09:05 Monday 2nd July 2012

A12.2
Reduced nutrition during early life has long-term effects on behavioural and metabolic responses to alarm pheromone in individual minnows

Shaun S. Killen (University of Glasgow, Scotland, UK) and Neil B. Metcalfe (University of Glasgow, Scotland, UK)

Nutritional deficits early in life can have lasting effects locomotor performance, but influences on other traits related to predator-avoidance are not known. We examined effects on the behavioural and metabolic responses to conspecific alarm pheromone (AP) in 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, controls exposed to open-field tests displayed increased caution when exposed to AP (minnow skin extract) compared to when the same fish were tested without AP. Compared to controls, food-deprived fish were less active in open-field tests – even without the presence of AP – and showed no change in behaviour when exposed to AP. When exposed to AP in respirometers, controls showed a decrease in metabolic rate (<50% of mean values when the same fish were exposed to water or swordtail skin extract), while food-deprived fish showed no change in metabolic rate during exposure to AP. Control individuals showing the greatest decrease in activity during open-field tests also showed the greatest decrease in metabolic rate while exposed to AP.

Results indicate that early food restriction decreases responsiveness to AP in minnows, by either affecting sensory ability or the motivation to respond to anti-predator cues. Such effects could be related to compensatory growth. Results also demonstrate a link between the metabolic and behavioural responses to AP in regularly growing individuals.

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09:50 Monday 2nd July 2012

A12.3
Social stress influences the physiological and cellular responses to thermal stress in fish

Suzanne Currie (Mount Allison University, New Brunswick, Canada), Sacha LeBlanc (Mount Allison University, New Brunswick, Canada) and Kathleen M. Gilmour (University of Ottawa, Ontario, Canada)

When resources are limited, animals in groups form dominance hierarchies with higher social status conferring fitness advantages while subordinate animals experience physiological costs, such as chronic stress, elevated metabolism and immunosuppression. We have previously determined that the formation of social hierarchies in juvenile salmonid fish results in a cellular stress response (i.e. the induction of heat shock proteins, HSPs). Thus, we were interested in determining whether this social stress influences how an animal copes with environmental stress such as heat shock. To this end, we evaluated the physiological and cellular stress response to a one-hour acute heat shock in juvenile rainbow trout in dominance hierarchies.

Social status did not affect the stress hormonal response to heat shock; however, the induction of HSPs was inhibited in both dominant and subordinate fish. Using critical thermal maximum as an index of thermal tolerance, we then determined that subordinate fish were more thermally sensitive than their dominant counterparts, but this response was decoupled from the induction of HSPs.

We conclude that social stress affects how fish respond to increases in temperature with likely deleterious consequences for overall fitness.

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10:30 Monday 2nd July 2012

A12.4
Are king penguins stressed during respirometry experiments? A potential confound for energetics calibration studies

Astrid S.T. Willener (University of Roehampton, UK), Lewis G. Halsey (University of Roehampton, UK) and Yves Handrich (CNRS, France)

To measure the energy expenditure (EE) of free-ranging animals, proxies of EE are recorded using miniature data loggers, which typically require calibration of those proxies with EE in the laboratory. For example, king

penguins have been exercised on treadmills and in water tunnels while their rate of oxygen consumption is measured along with proxies on data loggers such as heart rate and overall dynamic body acceleration. Such studies are arguably stressful to the subject animals, however, and if so this could invalidate the calibrations obtained. To date, no studies have quantified whether and how energetic-proxy calibrations are influenced by short-term stress-induced physiological changes in any animal.

The short-term effect of stress on EE, heart rate and dynamic body acceleration in king penguins was investigated during sham calibration experiments. These experiments involved two conditions: high activity (walking on a treadmill), and low activity (resting).

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11:00 Monday 2nd July 2012

A12.5
Accelerometry for estimating behaviour and metabolic rate in fish

Serena Wright (Swansea University, Wales, UK), Julian Metcalfe (Centre for Environment Fisheries and Aquaculture Science, UK), Stuart Hetherington (Centre for Environment Fisheries and Aquaculture Science, UK) and Rory Wilson (Swansea University, Wales, UK)

The ability to define and quantify the behaviour and energetic costs of different activities is fundamental to a full understanding of fish ecology and spatiotemporal movement. Recoding metrics of the behaviour and physiology of individual fish outside the laboratory or in large homogeneous groups, such as those encountered in aquaculture systems, is problematic. New telemetry methods using archival tags that incorporate triaxial accelerometers promise to provide a repeatable and robust methodology that allows the combined measurement of fish behaviour and energetics. Fine-scale behavioural patterns can be monitored with triaxial accelerometers along with overall dynamic acceleration, which has already been shown to relate linearly to oxygen consumption in a range of terrestrial species and one species of elasmobranch.

We present data from European sea bass (*Dicentrarchus labrax*) tagged over a period of three months that show correlations between vectorial dynamic body acceleration and oxygen consumption during both spontaneous and steady-state swimming. Through behavioural studies with Atlantic cod (*Gadus morhua*) we also show how activity varies in response to changes in environmental conditions (enrichment and stocking densities).

Although there is still a need to further develop analytical methods for acceleration data, our results clearly show accelerometry to be a powerful tool for remotely monitoring fish activity over extended periods, allowing us to identify specific patterns of behaviour and assess the relationship between activity and oxygen consumption of fish in both aquaculture situations and at large in their natural habitat.

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11:15 Monday 2nd July 2012

A12.6
Maternal effect, corticosterone and the adaptive phenotype

Jean Clobert (CNRS, France)

Variation of corticosterone during pregnancy has revealed many effects on the offspring phenotype after birth, the effect which might persist at adulthood. Many of these variations have been interpreted as either malformation or the production of a sub-optimal phenotype. I will argue that in many cases these modifications turn out to be adaptive and will review some cases where such adaptations have been demonstrated to be advantageous. I will then examine potential effects on the overall population dynamics.

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11:30 Monday 2nd July 2012

A12.7
The implications of variation in metabolism and DGC expression in the speckled cockroach, *Nauphoeta cinerea*: Consequences for life history

Natalie G. Schimpf (University of Queensland, Australia), Philip G.D. Matthews (University of Queensland, Australia) and Craig R. White (University of Queensland, Australia)

Both metabolic rate (MR) and respiratory gas exchange patterns vary considerably among and within species. The variation in metabolism remains even once several biotic (e.g. size, age and sex) and abiotic (e.g. temperature and humidity) influences are taken into account. Despite a growing body of literature, there is no consensus on the life history consequences of metabolic variation, and the implications of differences in discontinuous gas exchange cycle (DGC) expression are even less apparent.

Here we present the findings from a lengthy body of research that broadly encompassed the examination of the life history consequences of variation in metabolism and DGCs with respect to a suite of fitness traits in an ectotherm, *Nauphoeta cinerea* (speckled cockroach). More specifically, our research focused on the effect of MR and DGC on survival during food and water restriction, the effect of MR and DGC on reproductive performance (number of offspring and gestation duration) for both males and females and the association between metabolic rate and several fitness traits (locomotor performance, dominance and cold tolerance) in males. The results for MR were context-dependent and MR was often only associated with the fitness traits through interactions with age and mass (although male MR was negatively associated with the average number of babies he produced). A positive correlation between DGC expression and survival demonstrated a fitness benefit of DGCs for the first time. The associations between DGCs and reproductive performance measures however are less clear cut, especially for males.

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12:00 Monday 2nd July 2012

A12.8
The energetics of gap-crossing in forest canopies: Parkour athletes as a model for arboreal apes

Samuel R.L. Coward (University of Birmingham, UK), Lewis G. Halsey (University of Roehampton, UK), Robin H. Crompton (University of Liverpool, UK) and Susannah K.S. Thorpe (University of Birmingham, UK)

Arboreal apes such as orang-utans face unique challenges negotiating complex forest environments containing gaps between trees. These gaps are shortest at the terminal branches of tree crowns, yet here the branches are also at their thinnest and most compliant. Orang-utans can theoretically bridge these gaps in a number of ways:

- jumping from one tree to another;
- utilizing compliance by swaying the tree they are in, oscillating the trunk back and forth until it bends sufficiently to allow the gap to be bridged; and

• descending to the ground, crossing the gap and then ascending the second tree to the original height.

Mathematical models of these behaviours are presently available to estimate the costs of these different choices, but they are based on first principals alone and thus only address the external work done on the animal. Here we use Parkour athletes as a model for arboreal apes to determine the relative costs for these three gap crossing behaviours. Oxygen consumption was measured using a portable respirometry system and body movements were tracked by cameras.

Jumping a distance of 1.8 m requires the equivalent energy used to climb only a single metre of a vertically-placed ladder (an 'ideal tree'), while swaying a carbon fibre pole to cross the same gap was up to 12 times less costly than one metre of ladder climbing. These findings might partly explain the presence of tree swaying behaviour in orang-utans and why some individuals never leave the trees.

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13:20 Monday 2nd July 2012

A12.9
Effects of personality on spatial learning and memory in eastern rock sengis

Heike Lutermann (Mammal Research Institute [MRI], University of Pretoria, South Africa), Sasha Hoffmann (MRI, University of Pretoria, South Africa), Katharina Medger (MRI, University of Pretoria, South Africa) and Heike Lutermann (MRI, University of Pretoria, South Africa)

The evolution of consistent inter-individual differences in behaviours or personality has recently become the focus of many studies. Personality traits such as exploratory behaviour can affect space use and dispersal in wild populations, rendering them important for ecological research. More proactive animals are often assumed to roam further and thus have access to additional food sources. They might also, however, face a higher predation risk than reactive individuals. Similarly, personality traits could be expected to affect learning abilities in spatial tasks. We tested this hypothesis in the eastern rock sengi (*Elephantulus myurus*) by measuring exploratory behaviour, boldness and aggressiveness in wild as well as captive sengis across time and contexts.

Animals were trained to retrieve a hidden food item in an open plane arena and possible correlations between personality traits and spatial learning were evaluated. We observed strong correlations of exploratory, bold and aggressive behaviours in both wild and captive individuals that were consistent across time, indicative of a behavioural syndrome. Captivity did not affect personality and contrary to our hypothesis learning performance was not influenced by personality. In contrast, proactive (i.e. bolder, more exploratory and aggressive) individuals entered the arena faster and found food faster than reactive individuals.

Our findings suggest that personality-related behaviours are comparable between captive and wild animals and they are not correlated with learning but with the approach to spatial tasks. Consequently, proactive and reactive animals might be equally able to learn food locations, but the proactive approach could increase food encounter rates.

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14:00 Monday 2nd July 2012

A12.10
On the energetics of personality: From laboratory mice to wild chipmunks

Vincent Careau (University of California Riverside, California, USA), Theodore Garland Jr. (University of California Riverside, California, USA), Denis Réale (Université du Québec à Montréal, Quebec, Canada), Murray M. Humphries (McGill University, Quebec, Canada) and John R. Speakman (University of Aberdeen, Scotland, UK)

Physiologists are intrigued by the two- to three-fold variation among individuals in metabolic rate, even when at rest. Similarly, behavioural ecologists are attempting to explain why animals consistently behave differently from each other, i.e. exhibit personality. In this presentation, we address whether the large variation in energy expenditure is associated with such behavioural traits as aggressiveness, boldness and exploration in a novel environment. The answers to these questions allow us to cast a new light on an old field of research (energetics), while contributing to our understanding of the energetic consequences of animal personality.

The relationship between personality and metabolism (including resting metabolic rate and daily energy expenditure) has now been tested in a variety of animals, both in the laboratory and in the field, and at different levels of biological variation (among individuals, breeds and species). The variety of results obtained reveals the complexity of personality–metabolic rate relationships and calls for the need to integrate concepts and measure of performance into the 'pace-of-life' syndrome hypothesis.

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14:15 Monday 2nd July 2012

A12.11
Exercise changes personality

Frank Seebacher (University of Sydney, Australia), Elektra L.E. Sinclair (University of Sydney, Australia), Carolina R. Noronha (University of Sao Paulo, Brazil) and Ashley J.W. Ward (University of Sydney, Australia)

Personality, or consistent behavioural differences between individuals, is typically considered to be stable across contexts and over time. If personality constrains behavioural plasticity, individuals might be limited in their ability to respond adequately to environmental change, which would have profound evolutionary and ecological implications. Exercise is known to modify the physiological functions underlying behaviour, which leads to the question whether exercise influences personality and, if so, how?

We show that exercise significantly increases boldness, exploration and aggression in fish (*Gambusia holbrooki*). We also show that changes in exploration and aggression, but not boldness, are mediated by increased athletic performance, and that all changes are reversible when animals revert to a sedentary lifestyle. Hence, personality is not fixed within individuals, and does not necessarily limit the ability to respond to different challenges. Physiology plays a key role in mediating changes in personality in response to environmental variation.

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15:00 Monday 2nd July 2012

A12.12
Correlated evolution of behaviour and physiology in a multidirectional artificial selection on a wild rodent, the bank vole

Pawel Koteja (Institute of Environmental Sciences [IES], Jagiellonian University, Poland), Katarzyna Baliga-Klimczyk (IES, Jagiellonian University, Poland), Katarzyna M. Chrzascik (IES, Jagiellonian University, Poland), Geoffrey Dheyongera (IES, Jagiellonian University, Poland), Mateusz Konczal (IES, Jagiellonian University, Poland), Uttaran Maiti (IES, Jagiellonian University, Poland), Agata Rudolf (IES, Jagiellonian University, Poland), Clare Stawski (IES, Jagiellonian University, Poland) and Edyta T. Sadowska (IES, Jagiellonian University, Poland)

Experimental evolution offers a powerful yet underutilized approach to studying the connections between animal behaviour and physiology.

We established an artificial selection experiment, with lines of bank voles *Myodes* (*Clethrionomys*) *glareolus* selected for high swim-induced aerobic metabolism (A), the ability to maintain body mass on a low-quality herbivorous diet (H), intensity of predatory behaviour towards crickets (P), and unselected control lines (C). Four replicate lines are maintained in each direction.

In generation 11, voles from the A selected lines achieved 49% higher rate of oxygen consumption during swimming, and after a relaxed selection in generation 12, the difference in generation 13 was almost the same (48%). Voles from H lines maintained body mass in a test with low-quality diet better than those from C lines, but the values varied widely between generations (gen. 11 C: -0.35, H: -0.04 g/d; gen. 12 C: -0.32, H: +0.23 g/d; and gen. 13 C: -1.1, H: -0.68 g/d). In generations 11–13, 75–84% voles from the P lines attacked a cricket in at least one of two trials, whereas in C lines the proportion of 'predatory' voles was only 5–17%. Several other traits differed among the lines, both morphophysiological (basal and maximum metabolic rates, mass of organs, fat content, food consumption, thermogenic capacity, body temperature and reproductive effort) and behavioural (activity level and pattern, male dominance aggression and food preference).

The selected lines provide a unique model that can be used to study genetic correlations between physiological and behavioural traits, as well as cellular, biochemical and molecular factors underlining the correlations.

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15:35 Monday 2nd July 2012

A12.13

Seasonal patterns of body temperature daily rhythms in group-living Cape ground squirrels, *Xerus inauris*

Michael Scantlebury (Queen's University Belfast, Northern Ireland, UK), Marine Danek-Gontard (Queen's University Belfast, Northern Ireland, UK), Philip W Bateman (University of Pretoria, South Africa), Nigel C. Bennett (University of Pretoria, South Africa), Mary-Beth Manjerovic (University of Central Florida, Florida, USA), Kenneth E. Joubert (University of Pretoria, South Africa) and Jane M. Waterman (University of Manitoba, Winnipeg, Canada)

Organisms respond to cyclical environmental conditions by entraining their endogenous biological rhythms. Such physiological responses are expected to be substantial for species inhabiting arid environments that incur large variations in daily and seasonal ambient temperature (T_a). We measured the daily core body temperature (T_b) rhythms of Cape ground squirrels inhabiting an area of Kalahari grassland for six months from the Austral winter through to the summer.

Squirrels inhabited two different areas, an exposed flood plain and a nearby wooded shady area and occurred in different social group sizes, defined by the number of individuals that shared a sleeping burrow. There were significant changes in mean T_b and T_b acrophase over time, with mean T_b increasing and T_b acrophase becoming earlier as the season progressed. Squirrels also emerged from their burrows earlier and returned to them later over the measurement period. Large increases in T_b, sometimes in excess of 5°C, were noted during the first hour post emergence, after which T_b remained relatively constant. In addition, greater amplitudes of variation in T_b were noted in individuals inhabiting the flood plain compared with the woodland. Finally, there were significant effects of age and group size on T_b with a lower and less variable T_b observed in younger individuals and those from larger group sizes.

These data indicate that Cape ground squirrels have a labile T_b that is sensitive to a number of abiotic and biotic factors and presumably enables them to be active in a harsh and variable environment.

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15:50 Monday 2nd July 2012

A12.14

Effects of environmental stress and predator threat on the expression of bold and shy behaviour in the rainbow trout, *Oncorhynchus mykiss*

Lynne U. Sneddon (University of Chester, UK) and Jack S. Thomson (University of Liverpool, UK)

Animals exhibit a spectrum of individual behavioural traits that can be correlated through time or across contexts, and are termed personalities. One important personality measure is boldness, defined as a response towards novelty or risk, with bold and shy behavioural profiles often linked to physiological stress responsiveness. Theoreticians predict that these individual differences may be modulated through state and context-dependent conditions. Here the behavioural and physiological responses of bold and shy rainbow trout, *Oncorhynchus mykiss*, towards biotic (predation risk) and abiotic (temperature increase and hypoxia) environmental challenges were examined to determine whether risk or internal state altered the degree of boldness.

Bold and shy trout were classified according to their latency to approach a novel object. For the following 14 days, fish were exposed to either no, low (predictable) or high (unpredictable) risk via exposure to a simulated predator attack. For the final seven days of this study period, test subjects were either held in standard conditions or exposed to an increase in temperature or to a reduction in water oxygen content (50%) to simulate sudden environmental disturbances and to influence the energetic state of the fish. In response, bold fish generally became shyer, particularly under unpredictable threat, while shy fish became bolder except when facing a predictable threat. Risk and, for bold fish, higher temperature resulted in increased stress. These responses were linked with behavioural and physiological acclimation to environmental challenges and have important implications for populations facing environmental extremes due to anthropogenic activity and climate change.

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Poster Session – Sunday 1st July 2012

A12.15

Influence of predation risk and food availability on boldness in the rainbow trout, *Oncorhynchus mykiss*

Jack S. Thomson (University of Liverpool, UK), Phillip C. Watts (University of Liverpool, UK), Tom G. Pottinger (Centre for Ecology & Hydrology Lancaster, UK) and Lynne U. Sneddon (University of Chester, UK)

Boldness is an important behavioural trait defining responses to risk and novelty. Boldness is not fixed, however, and can change according to individual state or in response to context. The degree to which animals can modify behaviour might be linked with physiological stress responsiveness. Here we have examined how individual personality might influence the responses of bold and shy rainbow trout, *Oncorhynchus mykiss*, to variations in predation risk and food availability.

Individual fish were tested for boldness using a novel object paradigm: bold fish approached the object within 180 s and shy fish did not approach within 300 s. Subsequently, fish were exposed to either high (unpredictable), low (predictable) or no predation risk in addition to low (0.15% body-weight) or high (2% body-weight) food availability over seven days before being retested for boldness.

Bold trout were generally more behaviourally labile, becoming shyer in their latency to approach a novel object, whereas shy trout generally remained shy. The mRNA expression of three candidate genes selected for roles in boldness, stress physiology and appetite regulation generally increased in response to threat, particularly in shy fish. Plasma cortisol

concentrations, however, did not significantly change despite a peak in shy fish under predictable risk, possibly linking to divergence in the ability of bold and shy fish to predict regular events.

These data suggest fundamental differences in how bold and shy fish respond to environmental challenges and have important implications for the maintenance of particular behavioural phenotypes in the wild.

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Poster Session – Sunday 1st July 2012

A12.16
Links between personality and resting metabolism in the mouse

John R. Speakman (University of Aberdeen, Scotland, UK), Sarah Lee (University of Aberdeen, Scotland, UK) and Gernot Riedel (University of Aberdeen, Scotland, UK)

In recent years animal personality has attracted considerable attention due to its possible impact on ecological and physiological variation. In particular, personality traits such as boldness might be associated with some of the large individual variation in resting metabolic rate (RMR) that has been observed. In this study we investigated the relationship between 30 behavioural variables, obtained from three different personality tests:

- the open-field test;
- the elevated plus maze; and
- video analysis of behaviour following introduction to a novel cage environment.

We have shown previously that RMR is highly repeatable in mice. The measures of behaviour were also highly repeatable within individuals and correlated across the different personality tests. We used principal components analysis to summarize the behaviours of the mice, and then sought associations between scores on the principal component axes and RMR. There were significant relationships between RMR and the behavioural traits, suggesting that animals with low RMR were bolder. These relationships remained significant when the shared variation due to body mass was removed. Correlations between personality and metabolic rate might contribute to selection forces acting on individuals that keep the high level of observed variation in RMR.

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Poster Session – Sunday 1st July 2012

A13 – GENERAL ANIMAL BIOLOGY

A13.1
The evolution of vascular nitric oxide signalling

John A Donald (Deakin University, Australia), Melissa S. Cameron (Deakin University, Australia), Sofie Trajanovska (Deakin University, Australia), Edgar Liu (Deakin University, Australia), Leonard G. Forgan (Deakin University, Australia) and Susan L. Edwards (Appalachian State University, North Carolina, USA)

In mammals, the role of the endothelium as the primary source of nitric oxide (NO) that mediates vasodilation is well-established. Endothelium-derived NO is generated by an isoform of NO synthase (NOS) called NOS3. In addition, NO can be generated by two other NOS isoforms called NOS1 and NOS2. NOS1-containing nerves are called nitrergic nerves and activation of these nerves causes NO release. It is now clear that endothelial NO signalling is not ubiquitous in vertebrates, and in fact NO signalling arose in vertebrate blood vessels via nitrergic nerves. Comparative genomics have shown that NOS1 and NOS2 are present in all vertebrates, but the NOS3 gene has arisen in the tetrapod lineage. NOS3 first appears in amphibians, but NOS3 is not involved in endothelial NO signalling. Accordingly, a key question is when NOS3 first become expressed in the endothelium of tetrapods. Another important consideration is that NO is not a universal vasodilator of fish blood vessels with tetrapod-like NO-mediated vasodilation first appearing in teleost fish. This might be linked to the expression of the NO receptor, soluble guanylyl cyclase, in the vascular smooth muscle. In the absence of endothelial NO signalling in amphibians, NO control of blood vessels is provided by perivascular, nitrergic nerves. Interestingly, the majority of the nitrergic nerves have a very close association with sympathetic, adrenergic nerves, as NOS1 and tyrosine hydroxylase appear to be colocalized in many nerve terminals. In amphibians, however, NO-mediated vasodilation appears to operate independently of a functional adrenergic innervation.

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09:00 Monday 2nd July 2012

A13.2
Brain nitric oxide blockade increases blood pressure depending on peripheral alpha and beta adrenergic receptors in bullfrogs

Lucas A Zena (Universidade Federal de São Carlos/Universidade Estadual de São Paulo, Brazil), Luciane. H Gargaglioni (Sao Paulo State University, Brazil) and Kênia C. Bícego (Sao Paulo State University, Brazil)

We investigated whether nitric oxide acting on the brain of frogs presents an inhibitory tonus on arterial blood pressure (AP) and heart rate, as observed in mammals, by reducing the sympathetic activity dependent on alpha and/or beta adrenergic receptors during winter and spring/summer seasons. Body temperature and AP were measured by a telemetry device implanted into the abdominal cavity of the American bullfrog (*Lithobates catesbeianus*), with the catheter of the device inserted into the left aortic arch for AP and heart rate measurements. Additionally, blood cell flow was measured using a laser Doppler flowmeter sutured to the pelvic skin to calculate cutaneous vascular conductance. A guide cannula was implanted into the lateral ventricle of the brain for injections of L-NMMA, a non-selective nitric oxide synthase inhibitor, or mock cerebrospinal fluid (vehicle) and a PE cannula was inserted into the femoral vein for bolus injections of adrenergic antagonists, prazosin (alpha1) and sotalol (beta), and agonists, phenylephrine (alpha1) and isoproterenol (beta) or Ringer solution. Animals were maintained at 25°C during all the experiments. Mean AP, but not heart rate, was greater during winter than spring/summer. L-NMMA increased mean AP and decreased cutaneous vascular conductance, but did not change heart rate, during both seasons. The pretreatment with prazosin attenuated and sotalol accentuated the hypertensive effect of L-NMMA in both seasons.

We conclude that nitric oxide seems to act on the brain of frogs as a hypotensive agent via, at least in part, the inhibition of the sympathetic activity dependent on alpha vasoconstrictor and beta vasodilator adrenergic receptors.

Supported by FAPESP and CNPq.

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09:15 Monday 2nd July 2012

A13.3
Effects of nitric oxide on developmental haemodynamics in hypoxic-incubated embryonic White Leghorn chicken (*Gallus domesticus*)

Dane A. Crossley II (University of North Texas, Texas, USA), Tobias Wang (Aarhus University, Denmark) and Nina K. Iversen (Aarhus University, Denmark)

Oxygen availability during prenatal life is critical for normal maturation and hypoxia is associated with cardiovascular disease. Nitric oxide (NO) is a regulatory gas that is dependent on oxygen availability and *in situ* studies show an endothelium-derived, NO-mediated pulmonary and systemic arterial relaxation in embryonic chicken vasculature. We hypothesized that chronic hypoxic incubation (15% O_2) would reduce *in vivo* NO tone in embryonic chickens and dampen NO-mediated systemic hypoxic vasodilation compared to control embryos (21% O_2).

At days 15 and 19 of incubation, embryos were instrumented with a chorioallantoic arterial catheter for measurement of arterial pressure (P_m) and heart rate (f_H) to determine responses to drug injection. Day-19 embryos were also instrumented for measurement of femoral arterial blood flow (Q_f). Acute blockade of NO production via LNAME injection increased P_m for both days and oxic groups and the response intensity increased at day 19, but was similar between oxic groups. Injection of NO donor, sodium nitroprusside (SNP), decreased P_m, with the greatest response in control day 19 embryos. The intensity of P_m response to SNP was blunted in hypoxic embryos. LNAME injection intensified acute P_m response to 10% O_2 for days 15 and 19 and the oxic groups. It also reduced Q_f.

Collectively, data suggest that hypoxic incubation increases cardiovascular sensitivity to NO, but does not impact resting NO tone. While NO tone may be fixed, sensitivity to NO can be altered by developmental hypoxia. We must reject our hypothesis that chronic hypoxic development blunts NO tone as well as contributing to acute hypoxic vasodilation.

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09:30 Monday 2nd July 2012

A13.4
Oxygen binding by dwarf caiman haemoglobin that lacks key allosteric effector-binding sites

Roy E. Weber (Aarhus University, Denmark), Angela Fago (Aarhus University, Denmark), Hans Malte (Aarhus University, Denmark), Jay F. Storz (University of Nebraska at Lincoln, Nebraska, USA) and Thomas A. Gorr (University of Zurich, Switzerland)

Vertebrate haemoglobins commonly exhibit high intrinsic O_2 affinities that are reduced by allosteric effectors found in the red cells, such as protons, organic phosphates (ATP in ectotherms and diphosphoglycerate or 'DPG' in mammals) and chloride ions that bind at specific, charged amino acid residues of haemoglobin α and β protein chains. Adaptations in haemoglobins' O_2-transporting properties in response to variations in ambient O_2 availability or metabolic requirements are commonly mediated by changes in the concentration of the effectors or in the number of effector binding sites. We discovered that specific β-chain lysine and histidine residues that play key roles in allosteric regulation of the O_2 affinity of vertebrate haemoglobins (including hitherto-investigated crocodilians) are substituted by neutral amino acids in Dwarf caiman (*Paleosuchus palpebrosus*) haemoglobin. Aiming to elucidate the consequences of the unique combination of exchanges, its physiological implications and the possible compensatory adjustments to secure tissue O_2 delivery, we investigated O_2 binding of Dwarf caiman haemoglobin and its sensitivities to pH, CO_2, organic phosphates and chloride. We compared the data with those of abnormal human haemoglobin mutants that exhibit individual amino acid exchanges at the same sites and the crocodilian haemoglobins that have been functionally characterized. The study reveals novel mechanisms for maintaining allosteric regulatory potential in Dwarf caiman haemoglobin, even compared to the haemoglobins of other crocodilians (American alligator, Nile crocodile and Spectacled caiman).

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09:45 Monday 2nd July 2012

A13.5
The interaction effects between digestion, exercise and reproduction in the oviparous squamate, *Lamprophis fuliginosus*

Alexander G.S. Jackson (University of California Irvine, California, USA) and James W. Hicks (University of California Irvine, California, USA)

Physiological states interact in complex ways. Historically, physiological states have been studied in isolation; however this is not necessarily how an animal experiences this type of physiological challenge in an ecological setting. Common physiological challenges include the elevated metabolic states of digestion, exercise and reproduction.

We chose to study a tropical oviparous snake that can produce up to six clutches of eggs in one year. This species continues to consume meals up until the late stages of gravidity; hence it is a strong model system for examining the interaction effects between digestion, exercise and reproduction. The goal of our research was to tease apart the interaction effects between gravid and non-gravid females during exercise, digestion and postprandial exercise.

We compared the energetic costs of rest, gravidity, digestion, exercise and postprandial exercise using respirometry at 25 ±1ºC. Our results show that digestion and exercise have metabolic scopes up to 6.0 and 10.0, respectively, whereas gravidity has a metabolic scope of just 1.3. Furthermore, during postprandial exercise there is a prioritization towards the digestive process, with a metabolic scope intermediate between digestion and exercise. When a gravid animal is subjected to postprandial exercise, however, we do not detect any interaction effect.

From these results, we conclude that both gravid and non-gravid females demonstrate a prioritization towards digestion during postprandial exhaustive activity and that in this species the energetic cost of reproduction is not detectable when combined with either digestion or exercise.

This work was partially supported by NSF Grant IOS 0922756 to JWH.

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10:30 Monday 2nd July 2012

A13.6
Effect of heterospecific competitors on the link between standard metabolic rate and growth performance in stream-living juvenile salmon

Donald Reid (University of Glasgow, Scotland, UK), Neil B. Metcalfe (University of Glasgow, Scotland, UK) and John D. Armstrong (Marine Scotland Science, UK)

A range of laboratory experiments have suggested that inter-individual variation in energy budgets impacts greatly on performance, but this influence becomes less clear when examined in systems that better reflect natural conditions. Standard metabolic rate (SMR) in Atlantic salmon (*Salmo salar*) is positively related to dominance status and ability to obtain a territory. However, it is not apparent, however, how this is affected by competition from heterospecifics that would be expected to occur in the wild, such as brown trout, *Salmo trutta*.

The relationships between estimated SMR, dominance and growth rates of yearling Atlantic salmon were examined under different trout densities using replicate sections of a large-scale controlled experimental stream. The SMR of salmon was strongly correlated with their dominance rank, but was not correlated with growth rate when in the absence of trout. Moreover, at low trout densities salmon demonstrated a negative relationship between SMR and growth, possibly because the trout had a disproportionate effect on higher-ranking salmon, disrupting their territorial status and allowing low SMR (subordinate) individuals to perform better. Trout interacted with each other more at a higher density, however, leading to a positive SMR-growth relationship in the salmon. The relative performance benefit of a high SMR is thus conditional on the presence and density of heterospecific populations, demonstrating the ecological importance of interspecific effects on intraspecific physiological traits.

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10:45 Monday 2nd July 2012

A13.7
Cost of surfacing in the air-breathing striped catfish *Pangasianodon hypophthalmus*

Sjannie Lefevre (Aarhus University, Denmark), Tobias Wang (Aarhus University, Denmark), Do T.T. Huong (Can Tho University, Vietnam), Nguyen T. Phuong (Can Tho University, Vietnam) and Mark Bayley (Aarhus University, Denmark)

Due to the increased travelling distance involved when an air-breathing fish has to swim to the surface and down again, it can be proposed that air-breathing would result in a rise in the cost of transport (COT). In order to investigate this hypothesis, we used the striped catfish *Pangasianodon hypophthalmus*, which has a capacity for aquatic oxygen uptake in normoxia that is sufficient to support metabolism during swimming without air-breathing, allowing for a comparison of COT in fish swimming with and without surfacing. We measured the partitioning of oxygen uptake (M_{O2}) and calculated the net COT with and without access to air at all swimming speeds up to the critical swimming speed. The net cost of transport was 25% lower in fish that did not air-breathe compared to fish that did, showing that the energetic cost of surfacing can be substantial.

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11:00 Monday 2nd July 2012

A13.8
Assessing environmental adaptation ability in fish

Guy Claireaux (University of Brest, France)

Challenge tests are frequently used by clinicians to evaluate the cardio-respiratory performance of humans. The main outcomes of such tests are information about maximum oxygen consumption and heart rate, recovery capacity, power, velocity, anaerobic and breathlessness thresholds. Dysfunctions that are potentially difficult to detect in resting conditions can also be revealed. Interestingly, a parallel can be drawn between human medicine and environmental physiology in that in both cases the question addressed is whether an animal (whether human or not) is fit to do what it has to do in order to survive.

In both fields, cardiovascular and respiratory performances are considered as indicators of fitness in its most literal sense. Contrary to human medicine, however, relatively few attempts have been made to use challenge tests to relate current mechanistic understanding of fish physiology to their performance in the field. Yet, issues such as preserving exploited fish stock, improving aquaculture practices, controlling alien species, protecting marine biodiversity and assessing the impact of toxicant accumulation in aquatic ecosystem would all benefit from physiologically-sound interpretations of fitness. In this context, the objective of this presentation is to revisit various aspects of performance in fish in order to highlight their links with the parent notions of functional integrity, robustness and fitness.

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11:15 Monday 2nd July 2012

A13.9
Getting the most out of a mussel: Determining aerobic scope in the freshwater painter's mussel (*Unio pictorum*)

Glenn J. Lurman (University of Bern, Switzerland)

According to the oxygen limitation of thermal tolerance model, an animal's upper thermal limit is determined by its maximal oxygen consumption (M_{O2max}), and its aerobic scope as a consequence. Both are readily determined in active animals, however their determination in sessile animals is challenging due to their sessile nature. I employed three methods for determining M_{O2max}, and ultimately aerobic scope in the mussel *Unio pictorum* to assess any influence the method used may have on M_{O2max} and/or aerobic scope.

In the first experiment, summer acclimatized (24ºC) mussels (19.3 ±2.1 g) were placed in a respirometer with a silt substrate for burrowing. Three parameters were determined using this set-up:

- first, the M_{O2max} during burrowing; 0.79 ±0.13 mg O_2 h^{-1};
- second the M_{O2max} immediately following voluntary valve closure; 0.86 ±0.07 mg O_2 h-1; and
- third, the resting metabolic rate; 0.47 ±0.06 mg O_2 h^{-1}.

In a second experiment summer-acclimatized (24ºC) mussels (19.3 ±1.7 g) were heated by 2°C d^{-1} to 36°C to determine the M_{O2max} after acute heating. The maximum M_{O2} attained was 0.76 ±0.11 mg O_2 h^{-1} at 30ºC. There was no statistically significant difference. There was generally good agreement between these parameters, where they were 1.7, 1.8 and 1.6 times above the resting M_{O2} at 24°C, respectively.

Thus, M_{O2max} and aerobic scope can be reliably determined in U. pictorum using these three methods, lending support to the oxygen limitation of the thermal tolerance model that predicts thermal limits are set by the aerobic scope.

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11:30 Monday 2nd July 2012

A13.10
Respirometry, doubly-labelled water turnover and accelerometry – comparing their accuracy and applicability for measuring energy expenditure in northern fur seals

Alex J.M. Dalton (University of British Columbia**,** British Columbia [BC], Canada), David A.S. Rosen (University of British Columbia, BC, Canada) and Andrew W. Trites (University of British Columbia, BC, Canada)

Accurate estimates of energy expenditure are essential for understanding the ecological interactions between marine predators and their prey. This includes understanding the potential influence of nutritional stress in declining marine mammal populations, such as the Pribilof Islands' northern fur seals (*Callorhinus ursinus*). Our objective was to compare measured rates of oxygen consumption to two alternative methods for measuring energy expenditure in wild animals: doubly-labelled water turnover (DLW) and two- and three-dimensional accelerometry.

Daily energy expenditure was measured for each method simultaneously in six sub-adult, captive, female fur seals over five-day trials four times per year. Oxygen consumption was measured continuously inside a metabolic chamber that enclosed a saltwater tank and associated haul-out area. Rates of oxygen consumption yielded average energy expenditure estimates of ~10,500 KJ/day. In comparison, the DLW method resulted in the overestimation of energy expenditure by 5–10%.

The ability of accelerometers to predict energy expenditure met with mixed results. Surprisingly, common partial overall dynamic body acceleration techniques did not result in strong predictive power. The simpler, less expensive, three-dimensional activity monitors, however, showed greater promise for predicting short- and long-term rates of energy expenditure. In addition to being cheaper and easier to compute than accelerometers that provide overall dynamic body acceleration estimates, these three-dimensional accelerometry monitors might provide activity-specific energetic expenditure estimates over shorter time frames than DLW methods. Further analyses are required to assess their effectiveness over different levels of activity and to determine the potential effect of seasonal changes in physiology on their predictive capability.

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11:45 Monday 2nd July 2012

A13.11
Can bar-headed geese potentially fly over Mount Everest?

Lucy A. Hawkes (Bangor University, Wales, UK), Patrick J. Butler (University of Birmingham, UK), Peter Frappell (University of Tasmania, Australia), Jessica U. Meir (University of British Columbia, British Columbia, Canada), William K. Milsom (University of British Columbia, British Columbia, Canada), Graham R. Scott (McMaster University, Ontario, Canada) and Charles M. Bishop (Bangor University, Wales, UK)

The bar-headed goose, *Anser indicus*, is an Asian species that carries out one of the most spectacular vertebrate migrations, travelling over the Himalayan mountains between wintering grounds at sea-level in India and breeding grounds in Northern Asia and the Tibetan Plateau. It has been considered a regular migrant at extreme high altitude (>9,000 metres) and has been the focus of much research attention since a report that climbers had seen them flying over the summit of Mount Everest.

In the only study of bar-headed geese exercising in low oxygen conditions, captive bar-headed geese were unable to sustain cardiac output in severely hypoxic conditions (7% O_2) compared with normoxia, suggesting that the delivery of oxygen to the tissues might have been centrally limited. This consequently casts doubt on the ability of the

bar-headed goose to fly at extreme altitude and is a paradox that has remained unresolved.

In this study, we set out to investigate the exercise performance of bar-headed geese in simulated high altitude conditions. All captive bar-headed geese successfully completed running trials (maintained station for 15 minutes) in normoxia and in severe hypoxia with no signs of fatigue. Values describing heart rate, V_{O2}, V_{CO2} and blood gases were collected and demonstrated that geese can sustain cardiac output similar to flight in order to cope with the challenges of exercise in hypoxia. We also model the maximum altitude at which a bar-headed goose might expect to be found flying aerobically and compare it to GPS tracking data from wild geese.

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12:00 Monday 2nd July 2012

A13.12
Flying high on sugar: Carbohydrate oxidation kinetics in the ruby-throated hummingbird (*Archilochus colubris*)

Chris Chen (University of Toronto, Ontario, Canada) and Ken Welch (University of Toronto, Ontario, Canada)

Nectarivorous hummingbirds subsist almost exclusively on a mixture of sucrose, glucose and fructose found in floral nectar. Previous studies have shown that hummingbirds can fuel hovering flight (the most energetically expensive form of vertebrate locomotion) almost exclusively using recently ingested sucrose. In mammals, the relative ability of glucose and fructose (components of sucrose) to fuel aerobic exercise is limited by how quickly each hexose is transported to and oxidized in muscles.

The relative capacities for the direct utilization of glucose and fructose by hovering hummingbirds remain unknown. To examine the use of glucose and fructose, ^{13}C-enriched solutions of each sugar were administered separately. $^{13}C/^{12}C$ ratios in expired CO_2 were measured via capture of exhaled breath samples using feeder-mask respirometry and subsequent mass spectrometric analysis. Similar to previous studies involving rufous and Anna's hummingbirds, we found hovering ruby-throated hummingbirds' (*Archilochus colubris*) transition from exclusively oxidizing endogenous fatty acids (RQ≈0.71) when fasted to oxidizing newly-ingested carbohydrates (RQ=1.0) within 40 to 60 minutes of access to either glucose or fructose solutions. Interestingly, the percentage of metabolism supported by each hexose was found to be similar. Furthermore, the rate of nectar intake, fractional turnover of stable carbon isotope signatures and energy expenditure were in general higher when ingesting fructose than glucose.

These results confirm that dietary sugars are the main fuel for hovering hummingbirds but suggest there are behavioural differences in how hummingbirds assimilate and metabolize fructose and glucose.

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12:15 Monday 2nd July 2012

A13.13
Does Na^+/K^+-ATPase set the critical thermal minimum of *Drosophila*?

Heath A. MacMillan (University of Western Ontario, Ontario, Canada), James F. Staples (University of Western Ontario, Ontario, Canada) and Brent J. Sinclair (University of Western Ontario, Ontario, Canada)

The insect critical thermal minimum (CT_{min}) is associated with a failure of ion transport that ultimately disrupts neuromuscular excitability. We hypothesized that the insect CT_{min} is determined by temperature effects on the activity of primary ion-pumping enzymes. Here, we used 26 *Drosophila* species with >10ºC range in CT_{min} to examine the relationship between the CT_{min} and temperature effects on Na^+/K^+-ATPase activity in a phylogenetically-independent manner.

The temperature-activity relationship of Na^+/K^+-ATPase was measured using a ramped spectrophotometric assay technique, allowing entire curves of Na^+/K^+-ATPase activity to be generated from individual samples. *Drosophila* species that originated from higher latitude locations had lower CT_{min}, and higher Na^+/K^+-ATPase activity at low temperatures. Interestingly, differences in Na^+/K^+-ATPase activity at low temperatures among species appear to be driven by alterations in the position of the temperature–activity curve, rather than changes in thermal sensitivity (Q_{10}).

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13:30 Monday 2nd July 2012

A13.14
Effect of cold acclimation on troponin I isoform expression in striated muscle of rainbow trout

Todd E. Gillis (University of Guelph [UoG], Ontario, Canada), Sarah L. Alderman (UoG, Ontario, Canada), Jordan M. Klaiman (UoG, Ontario, Canada) and Courtney A. Deck (UoG, Ontario, Canada)

In vertebrates each of the three striated muscle types (fast skeletal, slow skeletal and cardiac) contain distinct isoforms of a number of different contractile proteins including troponin I (TnI). The functional characteristics of these proteins have a significant influence on muscle function and contractility. The purpose of this study was to characterize which TnI gene and protein isoforms are expressed in the different muscle types of rainbow trout, *Oncorhynchus mykiss*, and to determine whether isoform expression changes in response to cold acclimation (4ºC).

Semi-quantitative real-time polymerase chain reaction was used to characterize the expression of seven different TnI genes cloned from Atlantic salmon (*Salmo salar)* and rainbow trout. One-dimensional gel electrophoresis and tandem mass spectrometry were used to identify the TnI protein isoforms expressed in each muscle type.

Interestingly, the results indicate that each muscle type expresses the gene transcripts of seven TnI isoforms. There are significant differences, however, in the expression pattern of these genes between muscle types. In addition, cold acclimation was found to increase the expression of specific gene transcripts in all muscle types. The proteomics analysis demonstrates that fast skeletal and cardiac muscles contain three TnI isoforms, while slow skeletal muscle contains four. No other vertebrate muscle to date has been found to express as many TnI protein isoforms.

Overall this study underscores the complex molecular composition of teleost striated muscle and suggests there is an adaptive value to the unique TnI profiles of each muscle type.

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13:45 Monday 2nd July 2012

A13.15
Coadaptation of thermal biology: A newt tale

Lumir Gvozdik (Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Czech Republic)

The coadaptation hypothesis between thermoregulatory behaviour and thermal performance curves constitutes the core of evolutionary thermal biology. Despite the long-held notion about the coadaptation of mean preferred body temperatures (T_p) and optimal temperatures (T_{op}) for organismal performance, empirical studies have demonstrated that the mismatch between both traits is common. Currently, two models based on imperfect thermoregulation and the selective advantages of higher body temperatures were proposed to explain this phenomenon.

Based on a 10-year survey of thermal biology in European newts, I

present results showing an 8–10°C mismatch between T_p and T_{op} that is unexplainable by the available theory. In the most thoroughly studied species, *Ichthyosaura alpestris*, various performance and life history traits showed disparate thermal sensitivities in which thermal compromise roughly matched mean T_p in both larval and adult stages. In turn, newt thermal requirements measured in the lab approached a local phenotypic optimum in their natural aquatic habitat.

I propose that T_p is coadapted with thermal compromise of disparate thermal performance curves rather than with their T_{op}. Future studies on the coadaptation between behavioural thermoregulation and thermal physiology will strive to focus on the thermal sensitivity of traits related to all major fitness components – growth, mortality and fecundity.

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14:00 Monday 2nd July 2012

A13.16

Heat shock protein 70 response to low temperature and elevated carbon dioxide in the false codling moth, *Thaumatotibia leucotreta*

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In insects, the cross tolerance and biochemical interactions between the various stresses of modified gas conditions and temperature can either elicit or block low temperature stress responses. The modes of action as to how gases can potentiate or reduce lethal low temperature stress are unknown, although it is thought that they potentially interfere with the biochemical mechanisms of low temperature tolerance, including up-regulation of proteins, the synthesis of cryoprotectants and remodelling of biological membranes. Few studies have examined the biochemical mechanisms and potential hypotheses explaining such interactions.

Here, using final instar larvae of the false codling moth (*Thaumatotibia leucotreta*) as a model organism, we attempt to differentiate between the possible mechanisms of action whereby gas treatments affect low temperature tolerance. Larvae were exposed to a range of temperature conditions (0ºC, 25ºC and 35ºC), high carbon dioxide (6% CO_2) and low oxygen (2% O_2) treatments separately as well as in combination for various durations prior to a standard low temperature exposure at -1ºC. At different time points during these experiments, larvae were assayed for heat shock protein 70, cryoprotectant expression levels and membrane lipid composition. Preliminary results indicate that heat shock protein 70 changed significantly over the course of and between the experiments.

Together with mortality data, this work provides insights into the molecular responses elicited during these treatments. In addition, these results also serve to identify potential cross-tolerance between the controlled atmospheres and temperature. This knowledge might lead to improved treatments for the control of pest insects, and *T. leucotreta* specifically.

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14:15 Monday 2nd July 2012

A13.17

Thyroid hormone regulates muscle phenotype during cold acclimation in zebrafish (*Danio rerio*)

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In zebrafish (*Danio rerio*), thyroid hormone (TH) plays a pivotal role in the acclimation of locomotive performance (sprint speed and critical swimming speed) with temperature. While TH is a principle regulator of metabolism in mammals, its contribution to locomotive performance during cold acclimation in fish is not explained by metabolic capacity. Our earlier work shows that at cold temperatures TH-regulated gene expression is tissue-specific, with important transcription factors such as PGC1α and PGC1β significantly up-regulated in skeletal muscle. Thus, we were interested in whether

TH regulates muscle physiology directly to maintain swimming performance at lower temperatures. To determine the effects of TH on muscle phenotype, we used propylthiouracil and iopanoic acid to induce hypothyroidism in zebrafish over three weeks' acclimation to 18˚C or 28˚C. We further supplemented hypothyroid treatments with 3,5-diiodothryronine (T_2) or 3,5,3'-triiodothyronine (T_3) to determine the specific role(s) of each TH metabolite on muscle function.

Transcription profiles and enzyme assays indicate that both T_2 and T_3 act to shift muscle phenotype by regulating the expression of muscle-specific enzymes such as the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and modulators such as phospholamban (an inhibitor of SERCA) and by altering their isoform compositions. We show that in addition to regulating development and metabolism in vertebrates, TH also regulates muscle physiology in ways that affect locomotive performance.

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14:30 Monday 2nd July 2012

A13.18

The efficiency of vascular counter-current heat exchange arrangements in endothermic fishes

Hans Malte (Aarhus University, Denmark), Jess Boye (Grenaa Gymnasium, Denmark) and Jess Boye (Grenaa Gymnasium, Denmark)

Endothermic fishes, like species of tuna and lamnid sharks, have elevated muscle temperatures compared to the ambient water. This is the result of high metabolic heat production in combination with a low heat conductance. Elaborate, lateral and central heat-exchanger arrangements conserving heat produced in the red muscle are well described, and it has been assumed that these are the major sites for the conservation of heat that explains the low conductance. Recently, however, modelling of heat transfer in bigeye tuna has shown that high-efficiency heat conservation within the white muscle is also necessary to explain the observed temperature profiles of bigeye tuna cross sections.

Blood supply to the white muscle, however, is through much simpler structures like vascular bands or triads of alternating venoles and arterioles that are interspersed in the muscle tissue. How do these simple heat exchangers compare to the more elaborate arrangements conserving heat in red muscle, and are they capable of conserving heat with an efficiency high enough to explain the high white muscle temperatures? We have modelled heat transfer in different counter-current arrangements and find that the efficiencies of the triad and vascular band arrangements, at identical vascular dimensions, are somewhat lower than the more elaborate red muscle heat exchanger arrangement. These arrangements can still conserve heat with the efficiencies required to explain the temperature profiles recorded in bigeye tuna, however, and thus they are essential for the ability of this species to be endothermic.

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14:45 Monday 2nd July 2012

A13.19

Anoxia-mediated NMDA receptor-silencing resulting from mitochondrial permeability transition pore activation in anoxia-tolerant turtle neurons

Leslie T. Buck (University of Toronto, Ontario, Canada) and Peter Hawrsyh (University of Toronto, Ontario, Canada)

Mammalian neurons are anoxia-sensitive and rapidly undergo excitotoxic cell death when deprived of oxygen, mediated largely by calcium entry through N-methyl-D-aspartate receptors (NMDARs). This does not occur in anoxia-tolerant western painted turtle neurons, where a decrease in NMDAR currents is observed with anoxia. This occurs in response to a modest increase in cytosolic $[Ca^{2+}]$(cyt) and can be prevented by calcium chelation during anoxia. The aim of this study was to determine whether mitochondrial calcium release by activation of the mitochondrial permeability transition pore (MPTP) was responsible for the anoxia-mediated rise in $[Ca^{2+}]$cyt and the decrease in NMDAR currents.

NMDAR currents were measured in turtle pyramidal neurons using whole-cell patch-clamp techniques under normoxic and anoxic conditions. $[Ca^{2+}]$cyt was determined by fluorometric analysis using Oregon Green.

During anoxia, NMDAR currents decreased by 47% and $[Ca^{2+}]$cyt increased by 9%. Normoxic application of the MPTP opener atractyloside resulted in a 23% decrease in NMDAR currents and a 9% increase in $[Ca^{2+}]$cyt. Cyclosporine A, an MPTP inhibitor, blocked the anoxia-mediated increase in $[Ca^{2+}]$cyt. A mitochondrial uncoupler was used to release the mitochondrial calcium store during normoxia and anoxia and resulted in a 30% increase in $[Ca^{2+}]$cyt. These data indicate that the anoxia-mediated rise in $[Ca^{2+}]$cyt is due to opening of the MPTP and is sufficient to decrease NMDAR currents during anoxia. Furthermore, since a mitochondrial uncoupler releases additional calcium during anoxia, we speculate that the mitochondrial membrane potential decreases in a regulated fashion to a new set-point.

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15:30 Monday 2nd July 2012

A13.20
11β-HSD2 in the zebrafish brain

Sarah L. Alderman (University of Waterloo, Ontario, Canada) and Mathilakath M. Vijayan (University of Waterloo, Ontario, Canada)

11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) converts active glucocorticoids to their inactive derivatives and is essential for conferring aldosterone-specific actions in mineralocorticoid target tissues, and for protecting glucocorticoid-sensitive tissues during stress. Fish lack the capacity to synthesize aldosterone, so the functional role of 11β-HSD2 is less clearly defined. The purpose of this study was to understand the potential for cortisol regulation via 11β-HSD2 deactivation in the adult zebrafish brain, an important cortisol target organ.

A spatial map of 11βhsd2 expression was created using *in situ* hybridization, and revealed near-ubiquitous distribution in the brain including known cortisol target nuclei. This contrasts with the highly-restricted expression observed in mammalian brains. The capacity of cortisol to regulate 11β-HSD2 activity and expression was tested using an acute air exposure stressor. The conversion of ^{3}H-cortisol to ^{3}H-cortisone was increased significantly at one hour post-stress and remained elevated for 24 hours, despite a rapid recovery in whole-body cortisol. A moderate decrease in 11βhsd2 transcript abundance at 20 minutes post-stress might reflect transcription inhibition via putative glucocorticoid-response elements identified in the 11β-HSD2 promoter.

Taken together, the results of this study suggest an important role for 11β-HSD2 in mediating the effects of cortisol in the brain of zebrafish.

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15:45 Monday 2nd July 2012

A13.21
Mechanisms underlying changes in the brain water content of crucian carp, goldfish and trout during exposure to high external ammonia

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Unlike mammals, fishes are vulnerable to increased internal ammonia (hyperammonemia) following feeding or exposure to high external ammonia (HEA). Hyperammonemia normally leads to the glutamine synthetase (GS)-catalysed conversion of ammonia to glutamine in vertebrates, which is usually thought to detoxify ammonia. Glutamine accumulation has been implicated in ammonia-induced brain swelling in mammals by increasing the intracellular osmolarity and water uptake of astrocytes. The present study determined whether:

- ammonia caused brain swelling in ammonia-sensitive trout (*Oncorhynchus mykiss*) compared to more ammonia-tolerant crucian carp (*Carassius carassius*) and goldfish (*Carassius auratus*);
- accumulation of brain water during HEA was related to glutamine accumulation; and
- responses of the brain *in vivo* to hyperammonemia could be mimicked *in vitro* using fish brain cell lines.

Exposure of all three species to HEA resulted in significant, step-wise increases in brain water content, which increased by 30% in crucian carp brain at the highest total ammonia concentration (~22 mM). Moreover, increases in brain water were proportional to increases in plasma ammonia and reversed after recovery in ammonia-free water. Prior injection of trout and goldfish with the GS antagonist methionine sulphoximine reduced GS activity and glutamine accumulation by 90% and 80%, respectively, but brain swelling persisted during HEA exposure. Prior methionine sulphoximine exposure of trout and goldfish brain cell lines, which served as a proxy for astrocytes, had little effect on cell survival during HEA exposure.

We conclude that the fish brain does take-up water during HEA, but these effects are reversible and unrelated to glutamine accumulation.

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16:00 Monday 2nd July 2012

A13.22
Ventilatory responses of spiny dogfish (*Squalus acanthias*) to environmental hypoxia and ammonia, including ventilatory control by plasma ammonia

Gudrun De Boeck (University of Antwerp, Belgium) and Chris M. Wood (McMaster University, Ontario, Canada)

In mammals, elevated levels of plasma ammonia causes stimulation of respiration, and recently this has also been proven in teleost fish e.g. trout. Broad ranges of fish – from shark to trout – hyperventilate after a meal as part of the specific dynamic action. In shark this happens without a change in arterial pO_2 or pCO_2, the classical regulatory mechanisms for ventilation, but concomitant increases of PNH_3 and $[NH_4^+]$ have been observed.

Since sharks try to retain as much nitrogen as possible to support the synthesis of their main osmolyte urea, why would they let the levels of plasma ammonia increase unless it serves as a signal? This hypothesis has been tested by injecting spiny dogfish (*Squalus acanthias*) with ammonia as either NH_4HCO_3 *versus* $NaHCO_3$ or $(NH_4)_2SO_4$ *versus* Na_2SO_4 (to control pH and HCO_3^- levels respectively), or injecting controls with NaCl. Additionally, responses to environmental water ammonia exposure (by NH_4HCO_3) and hypoxia have been recorded.

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16:15 Monday 2nd July 2012

A13.23

Roles of crustacean hyperglycaemic hormone in ionic and metabolic homeostasis in the Christmas Island blue crab, *Discoplax celeste*

Lucy M. Turner (Plymouth University, UK), Simon G. Webster (Bangor University, Wales, UK) and Steve Morris (University of Bristol, UK)

There is a growing body of evidence suggesting that crustacean hyperglycaemic hormone (CHH) has osmoregulatory roles in crustaceans. Little is known, however, regarding hormonally-influenced osmoregulatory processes in terrestrial decapods. *Discoplax celeste*, a land crab endemic to Christmas Island (previously misidentified on Christmas Island as *Discoplax hirtipes*), is an ideal model with which to test this hypothesis, since it exhibits a marked seasonal dichotomous distribution depending on the availability of freshwater (rainfall) and can be considered an intermediate in the lineage of water to air-breathing crabs. Furthermore, since *D. celeste* undertakes a metabolically challenging seasonal breeding migration, seasonal changes in metabolic demands might also be superimposed or otherwise integrated with those associated with ionic homeostasis.

Molecular techniques established the presence of two functionally active CHHs in *D. celeste*. Field studies on Christmas Island demonstrated that the concentration of circulating CHH in the haemolymph of *D. celeste* fluctuated with respect to both season and increasing water salinity to which crabs were acclimated. The injection of each of these CHHs was found to increase Na^+ uptake at the gills as well as clearance, filtration and urine flow rate at the antennal gland on a seasonally specific basis. Experiments to examine any effect of CHH on gill Na^+/K^+-ATPase or V-ATPase activity proved inconclusive, however, indicating that the osmoregulatory mode of action of CHH remains to be fully characterized in *D. celeste*.

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16:30 Monday 2nd July 2012

A13.25

The evolution of bipedalism in mammals: No evidence that it conferred a reduction in walking costs

Lewis Halsey (University of Roehampton, UK)

Debates about the evolution of human bipedality sometimes include discussion on the energy costs of terrestrial locomotion of extinct and extant hominins. Comparative analyses of hominin transport costs conducted to date, however, have been limited and potentially misinforming, in part because they fail to consider phylogenetic history. In the present study, we compare the measured costs of pedestrian locomotion in humans, chimpanzees (which are likely similar locomotion-wise to the closest common ancestor of these two species) and the estimated costs for *Australopithecus afarensis* (an early bipedal hominin), to a database of locomotory costs for mammals.

Using data for 81 species of mammal, we tested for phylogenetic signals and then generated a prediction line for transport costs based on body mass. We then compared this prediction with published data for the net energy costs of running and walking in humans, and walking in *A. afarensis* and common chimpanzees.

The cost of human walking was 25% lower than predicted, while the cost of running was 27% higher. The cost of *A. afarensis* walking was 32% lower than predicted, and the cost of common chimpanzee quadrupedal walking was 33% higher. All of these data points fall within the 95% prediction interval for mammals, however, and the difference between humans and our closest living relative the common chimpanzee is comparable to differences between other similarly closely-related species. We therefore conclude that there is no evidence that bipedalism confers a reduction in energy costs of pedestrian locomotion in mammals.

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Poster Session – Sunday 1st July 2012

A13.26

Geographic variation among Colombian populations of *Drosophila starmeri*: Evidences of possible cryptic speciation

Laura N. Afanador Barajas (Universidad Central, Colombia), Andrew J. Crawford (Universidad de los Andes, Colombia) and Clara E. Quijano (Universidad de los Andes, Colombia)

Under allopatric models of speciation, geographic variation is a crucial first step in the divergence of a single population. The products of speciation can be difficult to detect when behavioural or morphological divergence is not pronounced, leading to the presence of cryptic species. The aim of this study was to examine whether *Drosophila starmeri* is a homogeneous evolutionary entity or whether it is composed of multiple distinct lineages.

We quantified geographic variation in four traits in three populations of *D. starmeri* and compared them with other cactophilic *Drosophila*. We examined cuticular hydrocarbons, wing morphometrics, reproductive isolation and mitochondrial DNA (mtDNA) sequences to quantify geographic variation, infer possible evolutionary forces and look for evidence of possible incipient speciation.

Phylogenetic analyses of mtDNA suggested that *D. starmeri* are not monophyletic. We found two clades: one associated to population of Camarones and Barichara grouped to *D. starmeri* and *D. venezolana* from Venezuela; and the second clade was associated to Tatacoa population. These two populations were similar to each other, yet distinct from populations of Tatacoa in wing morphology and cuticular hydrocarbons. Inter-populational crosses between lab strains showed that only Barichara × Camarones produced offspring. These two pairs of populations were the only pairwise comparisons to show evidence of gene flow in mtDNA. Although not differentiated in wing morphometrics, our genetic and behavioural data suggest that the Tatacoa population could represent a possible incipient species. Based on the combined data, we conclude that populations of *D. starmeri* contain possibly two cryptic species.

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Poster Session – Sunday 1st July 2012

A13.27

Effects of the organophosphorous pesticide diazinon on heat shock protein levels in the freshwater shrimp *G. pulex*

Salem S. Elwahaishi (Nottingham Trent University, UK), Alan J. Hargreaves (Nottingham Trent University, UK) and Chris Lloyd Mills (Nottingham Trent University, UK)

Diazinon is an organophosphorothioate pesticide and acaricide developed in the 1950s. It is used in various formulations as a sheep dip and to control insects in soil, plants, fruit and vegetable crops in agricultural and urban environments. The freshwater shrimp *Gammarus pulex* (Crustacea, Amphipoda) is an aquatic invertebrate that is an important component of freshwater ecosystems. It is frequently used in ecotoxicity testing as it is sensitive to a range of pollutants, widespread in fresh water bodies, and an important part of the food web. Most toxicity studies investigate active ingredients in pesticides, taking little account of the potential toxicity of other formulation components, but we have found that the formulation has a more potent effect on *G. pulex* survival than the active ingredient alone. The aim of the present study was to compare the effects of diazinon and the formulation on the expression of heat shock proteins (HSP) in *G. pulex.*

It was found that the level of HSP20 protein in post-9,000 g supernatant samples was significantly increased by exposure to diazinon and the commercial diazinon formulation; however, there was no significant effect of these agents on HSP60 levels, while diazinon alone but not the formulation significantly increased the level of HSP70 in the supernatant. In contrast, diazinon and the commercial diazinon formulation significantly increased the level of HSP70 in 9,000 g pellet samples. It is concluded that HSP protein levels in *G. pulex* might represent a useful molecular biomarker to monitor organophosphate-induced environmental stress.

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Poster Session – Sunday 1st July 2012

A13.28
Quantitative changes in the macroglial cell of the basal-lateral part of amygdala of the brain of adult rats after chronic barbiturate intoxication

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The problem with studying the structure of the central nervous system by using potent drugs and narcotics, including barbiturates, is one of the most urgent in modern medicine. The aim of this study was to investigate the quantitative changes of macroglia, of the lateral part of the basic amygdala glia brain, of mature rats after chronic intoxication by barbiturates. The study was carried out on 120 white male rats of mature age. The animals received phenobarbitone (30 and 70 mg/kg) or benzonal (35 mg/kg) for a period of seven, 15, 30 or 60 days.

It was found that chronic intoxication with barbiturates is accompanied by proliferation of glial cells. Sometimes the effects of neuronophagia were determined. We can assume that at the conditions of chronic intoxication by barbiturates, quantitative changes in glia were associated with the increasing metabolic needs of neurons. The mechanism of action of the latter affects and inhibits the metabolism of cytochrome P450. With increasing time (60 days), proliferation of glial elements significantly increased on the background of reparative changes in the neurons. Similar quantitative changes were observed in the experimental groups of animals that received benzonal, but the intensity of these changes was less.

Thus, the findings suggest that the degree of quantitative changes in the amygdala glia of a rat's brain depends on the type of drug (phenobarbitone), the dose (70 mg/kg) and the duration of the experiment (seven, 15, 30 and 60 days).

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Poster Session – Sunday 1st July 2012

A13.29
Behavioural changes in mice with low ambient extracellular glutamate levels

Elizabeth A. Langer (University of Illinois, Illinois) and David Featherstone (University of Illinois Chicago, Illinois)

Cystine-glutamate transporters (xCTs) are the primary determinant of extracellular glutamate in the brain. Extracellular glutamate has been proposed to regulate glutamate receptor function (both iGluRs and mGluRs), and therefore behaviour. To test whether xCT function regulates behaviour, we examined behaviour in xCT mutant mice. Specifically, we examined behaviour in two different knockouts of the xCT gene (*sut* and *xCT*) and their respective genetic controls (C3H/HeJ and C57BL/6J) in an eight-arm memory maze, an elevated plus arm maze, a rotarod task, and a spontaneous alternation task.

Female *sut* mice made fewer alternations and arm choices in the spontaneous alternation task. Male *sut* mice made fewer choices, but the same number of alternations in this task. The *sut* mice performed similarly to their controls in the eight-arm task, rotarod task and elevated plus arm maze (*sut* females made fewer arm choices in this task than their controls, but did not vary in the amount of time spent in open or closed arms). *xCT* mice performed similarly to their controls in the spontaneous alternation and rotarod tasks.

Female mice of both strains were tested in the spontaneous alternation task to see whether changes in hormone levels during the oestrus cycle explained the decreased alternation behaviour seen in *sut* mice. There was no relationship between sex hormones and reduced spontaneous alternation in either strain, leading us to conclude that the reduced arm choice and spontaneous alternation performance seen in *sut* females is not related to changes in hormone levels across the oestrus cycle.

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Poster Session – Sunday 1st July 2012

A13.30
Does experience change kinematics of prey capture in squamates? The example of P*ogona vitticeps*

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Feeding efficiency is a key factor explaining adaptive response in animals. Lingual prehension is one of the major modes of food capture in squamates. Briefly, the tongue is protracted or projected toward the prey, adheres to the prey and retracts it into the buccal cavity. As soon as the prey is bitten by the jaws, the transport phase begins.

Here, we compare the kinematics of lingual prehension in newborn and adult agamid *Pogona vitticeps* from the digitization of high-speed video records using standardized prey (a cricket of similar size related to the snout-vent length of the filmed lizard). Kinematic variables depicting head, jaw and tongue movements were statistically compared.

At both ages, gape cycle is divided into oscillatory and capture phases. The mean duration of both phases is longer in newborns. During the capture phase, time to maximal tongue protrusion and time to maximum gape increase are longer in newborns. These data were compared with histological properties of the foretongue that contacted the prey in newborn and adult lizards. All of these morphological and functional data are used to test whether capture behaviour is entirely genetically fixed or can be improved by individual plasticity in adult squamates using lingual prehension.

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Poster Session – Sunday 1st July 2012

A13.31
Drosophila king tubby (*ktub*) mediates light-induced rhodopsin endocytosis and retinal degeneration

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The *tubby* (*tub*) and *tubby-like protein* (*tulp*) genes belong to a small family of genes whose functions remain unclear. They are found in multicellular organisms including animals and plants. The C-terminus of Tub and Tulp proteins are highly conserved and defined as the Tubby domain. Mutations in tubby family genes cause disease phenotypes, such as retinal degeneration and obesity. This study shows that *Drosophila king*

tubby (*ktub*) participates in rhodopsin (Rh1) endocytosis in response to light stimulation.

Immunocytochemical analysis shows that Ktub is expressed in the rhabdomere domain in dark conditions. When flies receive light stimulation, the Ktub transloclates from the rhabdomere to the cytoplasm and the nucleus of the photoreceptor cells. Wild type photoreceptors form Rh1-immunopositive large vesicles shortly after light stimulation. In light-induced ktub mutants, the majority of Rh1 remains at the rhabdomere, and only a few Rh1-immunopositive large vesicles appear in the cytoplasm of photoreceptor cells.

To further investigate the role of *ktub* in Rh1 endocytosis, this study examines its ability to block norpA-mediated Rh1 endocytosis. Mutation of the *norpA* allele causes massive Rh1 endocytosis in light conditions. In *ktub* and *norpA* double mutants, however, Rh1 endocytosis is blocked in response to light stimulation. This study also shows that *ktub* and *norpA* double mutants rescue the light-induced *norpA* retinal degeneration. Deletion constructs further demonstrate that the Tubby domain in the Ktub protein is necessary for Rh1 endocytosis.

Together, these results delimit the novel function of Ktub in Rh1 endocytosis and retinal degeneration.

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Poster Session – Sunday 1st July 2012

A13.32
The cost of reproduction in mice selectively bred for high and low food intake: A test of the oxidative stress theory

Aqeel Al Jothery (Aberdeen University, Scotland, UK) and John Speakman (Aberdeen University, Scotland, UK)

Reactive oxygen species (ROS) are generated as a by-product of aerobic metabolism causing damage to macromolecules. As such they have been suggested to potentially drive life history trade-offs (oxidative stress theory). To test this theory, antioxidant capacity, and oxidative damage to macromolecules was assessed in tissues collected from mice selectively bred for high (H) or low (L) food intake that were either non-reproductive (NR, controls) or at peak lactation (reproductive, R). R mice had significantly lower levels of reactive oxygen metabolites as well as lower total antioxidant capacity in serum compared to NR mice (measured by d-ROMS and OXY test: $p<0.05$). NR and R mice differed significantly in the activity of enzymatic antioxidants. In liver, catalase and glutathione peroxidase activity was reduced in R mice compared to NR mice ($p<0.05$) while in brain Cat activity was increased ($p<0.05$). No significant differences in superoxide dismutase were observed ($p>0.05$). Protein carbonyls, a biomarker of protein oxidation, were significantly reduced in the brains of R mice, but not in liver. No differences in above variables were observed between H and L mice. These results indicate that while reproducing mice had lower enzymatic antioxidant activity in liver, this did not result in increased oxidative stress and in the brain oxidative stress appeared to be lower during reproduction. Additional biomarkers, measuring oxidative damage to DNA and lipids need to be assessed and may clarify whether oxidative stress is a cost of reproduction.

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Poster Session – Sunday 1st July 2012

A13.33
Rapid growth of avian parasitic embryos despite decreased gaseous exchange across the eggshell

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Obligate avian brood parasites lay all their eggs in host's nests and play no role in the incubation or rearing of their progeny. Some parasites, like *Cuculus cuckoos*, hatch before their hosts and the altricial chick evicts all other eggs and/or nestlings. A hypothesized but so far untested parasite adaptation is that cuckoo embryos develop quicker due to a higher porosity of the eggshell allowing greater gaseous exchange, facilitating rapid development. We compared the water vapour conductance ($G H_2O$) of common cuckoo eggshells and passerines available both from the present study and the literature. The cuckoo eggs had significantly lower $G H_2O$ than that of their host species, and when compared to all passerines combined. Thus, permeability of the eggshell does not contribute to the rapid development of the cuckoo embryo. We infer that the mimicry of cuckoo eggs to their hosts is purely visual, and the physiological trait of $G H_2O$ is cuckoo-specific.

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Poster Session – Sunday 1st July 2012

A13.34
The relative toxicity of palladium toward *Gammarus pulex*

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The increased use of platinum group elements (PGEs) in automobile catalysts has led to global concerns about the potential environmental impact of these metals. In freshwater habitats *Gammarus pulex* is widely distributed, numerically abundant and sensitive to numerous toxins. *G. pulex* is also a key prey organism for fish. These features have led it to be commonly used in ecotoxicity testing.

The aim of this study was to investigate the impact of palladium on the freshwater amphipod *G. pulex.* It was found that palladium affected the survival of *G. pulex* with a 96-hour LC_{50} of 0.52 mg/L (4.98 µM). The feeding rate of *G. pulex* was also significantly reduced after 72 hours of exposure to 0.1 mg/L Pd (0.96 µM) or higher concentrations.

Heat shock proteins (HSPs) can act as molecular chaperones that aid protein folding. Environmental stress can induce increased HSP expression. Following 24 hours of exposure to up to 0.5 mg/L Pd (4.79 µM), no significant alteration in the level of HSP70 were observed in the supernatant or pellet of the 9,000 g fraction of homogenized *G. pulex*. In contrast, the induction of HSP60, which was only observed in the 9,000 g pellet, increased significantly after 24 hours of exposure to 0.1 mg/L Pd or higher concentrations ($p<0.001$).

It can be concluded that the feeding rate and the expression of HSP60 in *G. pulex* are sensitive indicators of Pd exposure under these study conditions.

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Poster Session – Sunday 1st July 2012

A13.35
Effect of fermented moist feed on *Salmonella typhimurium* in chicken gut

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Salmonellosis is one of the diseases that are transmitted by poultry and poultry products to humans. Probiotics can used to reduce this disease in chickens (Ali Wali N., Beal, *J. Int. J. Probiotics Prebiotics*. 2011; 6: 193–197). In this study an *in vitro* model of the poultry digestive tract was used

to determine the survival of a probiotic strain of *Lactobacillus plantarum* and its effect on *Salmonella typhimurium* in simulated digestive system of chicken.

In vitro model: Stage 1 crop: pH 4.6 (80µl 37% HCl) for 45 minutes at 41.4ºC. Stage 2 gizzard and proventriculus: pH 2.5 plus 0.320 g pepsin for 90min at 41ºC. Stage 3 small intestine: pH 6.2 (with $NaHCO_3$) plus 0.320 g pancreatin and 0.220 g bile salts for 120 minutes at 41ºC. With the exception of *L. plantarum* for FMF, all bacteria were added at the beginning of stage 1. FMF was prepared by inoculating moist feed (1 feed: 1.2 water) with *L. plantarum* and incubating for 24 hours at 30ºC. *Lactobacilli* and *Salmonella* were enumerated at 0, 45, 135 and 285 mins. All treatments were applied in triplicate.

Addition of *L. plantarum* prevented the recovery of *S. typhimurium*, suggesting an inhibitory effect on *S. typhimurium*. It is likely that lactic acid present in FMF increased the inhibitory effect of *L. plantarum*.

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Poster Session – Sunday 1st July 2012

A13.36

Cortisol elevation in common carp, *Cyprinus carpio*, improves ammonia excretion capacity during swimming at different feeding regimes, but diverts energy use towards protein and anaerobic metabolism

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Teleost responses to elevated cortisol levels depend on the degree of stress and are species-specific. This study was designed to investigate the effect of cortisol elevation on the metabolic strategy of in *Cyprinus carpio* under different feeding and swimming regimes.

Carp were fed 0.5% or 3.0% body weight. Carp received no implant (control), a coconut oil implant (placebo) or a cortisol in coconut oil implant (250 mg cortisol per kg fish) and monitored at 12, 24, 72 and 168 hours post-implant intervals.

Cortisol implants significantly elevated plasma cortisol at 12, 24 and 72 hours post-implant and returned to basal level at 168 hours post-implant. This resulted in increased plasma glucose and lactate levels in all groups, but did not impair U_{crit}. Feeding and swimming increased oxygen consumption and the effect of cortisol was only observed in the first 24 hours post-implant. High branchial ammonia clearance rate at U_{crit} indicated an enhanced excretion capacity in cortisol-treated fish that resulted in reduced ammonia accumulation in plasma. Despite high protein usage during aerobic metabolism with a high ammonia quotient and protein mobilization in the liver and muscle of cortisol-treated fish, low plasma ammonia was maintained. Cortisol implants also triggered anaerobic metabolism with high plasma lactate accumulation when swimming.

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Poster Session – Sunday 1st July 2012

A13.37

Differences in specific dynamic action as a possible explanation for inter-individual variability in the spotted wolffish (*Anarhichas minor*)

Felix J Christen (Maurice-Lamontagne Institute, Quebec, Canada) and Denis Chabot (Maurice-Lamontagne Institute, Quebec, Canada)

We are currently investigating the hypothesis that individual differences in the characteristics of specific dynamic action (SDA) explain a part of the variability in growth performance of spotted wolffish (*Anarhichas minor*). SDA describes the increment of metabolic activity and the energetic costs associated with feeding, predominantly induced by the biotransformation of nutrients and *de novo* protein synthesis during and after digestion.

Intermittent-flow respirometry was used to measure the oxygen consumption (M_{O2}) of fish fed with a 2% body mass ration in order to determine several SDA characteristics. A previous growth trial allowed us to select fish with the highest and lowest specific growth rate (hSGR and lSGR, respectively). Both groups had the same standard metabolic rate.

Total SDA duration and time to reach maximum M_{O2} values were significantly lower in the fast-growing group (SDA: 91.96 ±1.88 hours; time to peak: 41.31 ±3.82 hours) compared to the slow-growing group (SDA: 109.94 ±7.33 hours; time to peak: 57.81 ±5.00 hours). Peak M_{O2} values (hSGR: 34.28 ±1.76 mg/h/kg; lSGR: 28.72 ±1.9 mg/h/kg) were significantly higher for the fast-growing group. This suggests that some wolffish need longer to digest a meal of the same size. Over time, this should result in a decrease of food ingestion, and thus a lower growth rate.

Our second objective, which we are currently working on, is to confirm that hypoxia reduces growth rate through the same mechanism, peak M_{O2} being lowered due to a decrease in aerobic scope, and an increment in duration as a result. So far, this has only been demonstrated in cod.

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Poster Session – Sunday 1st July 2012

A13.38

Influence of biotic and abiotic cues on the acclimation of preferred body temperatures in a predator–prey system

Radovan Smolinsky (Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Czech Republic) and Lumir Gvozdik (Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Czech Republic)

The ability to modify phenotypes in response to heterogeneity of the thermal environment represents an important component of an ectotherm's non-genetic adaptive capacity. Despite considerable attention being dedicated to the study of thermally-induced developmental plasticity, whether or not interspecific interactions shape the plastic response in both a predator and its prey remains unknown.

We tested several predictions about the joint influence of predator–prey scents and thermal conditions on the plasticity of preferred body temperatures (T_p) in both parties of this interaction, using a dragonfly nymph–newt larvae system. Dragonfly nymphs (*Aeshna cyanea*) and newt eggs (*Ichthyosaura alpestris*) were subjected to fluctuating cold and warm thermal regimes (7–12 and 12–22°C, respectively) and the presence/absence of predator or prey chemical cues. Preferred body temperatures were measured in an aquatic thermal gradient (5–33°C) over a 24-hour period.

Newt T_p increased with developmental temperature irrespective of the presence/absence of predator cues. In dragonflies, thermal reaction norms for T_p were affected by the interaction between temperature and prey cues. Specifically, the presence of newt scents in the cold regime lowered dragonfly T_p.

We concluded that predator–prey interactions influenced thermally-induced plasticity of T_p but not in a reciprocal fashion. The occurrence of frequency-dependent thermal plasticity might have broad implications for predator–prey population dynamics, the evolution of thermal biology traits, and the consequences of climate change within ecological communities.

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Poster Session – Sunday 1st July 2012

A13.39
Loss of activity of protein protectant polyphenol oxidase as a result of cropping and transportation of red clover and grasses

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Ruminants convert plant material into high-quality animal protein and in doing so are reliant on microorganisms in the rumen. Improving the efficiency of rumen processes is important to reduce the environmental impact of ruminant agriculture. Plant protein can be protected from microbial degradation, using polyphenol oxidase enzymes (PPOs) that form less accessible protein–quinone complexes.

Two separate experiments were carried out with four rumen-fistulated Holstein-Friesian cows. Red clover and low PPO mutant were offered alternately in experiment 1, and cocksfoot, perennial ryegrass and timothy were offered alternately in experiment 2; rumen down-boluses were collected and frozen.

Protein, PPO and proximate-analysis of nitrogen (%N) and fibre were measured. Experiment 1 revealed decreased %N from field to farm to down-bolus. Losses in %N were associated with losses of protein from field to down-bolus. The bolus-washing process resulted in nitrogen loss. PPO activity was lost from field to farm, transport and warm weather being the likely cause. This continued during down-bolus formation. An improved washing process eliminated losses. All grasses lost protein during down-bolus formation. Cocksfoot lost 90% of PPO activity from field to farm, perennial ryegrass retained activity from field to farm and timothy exhibited minimal activity. PPO is physically deactivated during cropping and transport in red clover and grasses (not perennial ryegrass). Lack of a correlation of PPO activity with recovered protein might be a result of a loss of PPO activity. This was subsequently reduced in a further experiment (cold-storage of red clover, reducing activity losses from 68–36%).

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Poster Session – Sunday 1st July 2012

A13.40
The centenary of Scott's polar tragedy: The basic physiology of death from a starvation diet

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One-hundred years ago, Captain Robert Falcon Scott set out across the Ross Ice Shelf in the Antarctic hoping to be the first person in history to reach the South Pole. He was travelling on foot. Although his team of five men succeeded in crossing the 900 miles from the edge of the continent to 90° latitude by early 1912, man-hauling their sledges carrying food and equipment for most of the trip and conducting valuable science en route, they died on the return journey.

From relatively recent man-hauling expeditions in the Polar Regions, particularly the crossing of Antarctica by Fiennes and Stroud in 1992/93, it is now recognized that Scott's albeit substantial rations were providing an inadequate number of calories given the energy his men were expending during their journey; they were on a starvation diet. Consequently, at least the last three of Scott's party to fall almost certainly died from the degenerating cycle of gradual emaciation leading to reduced strength to man-haul and reduced heat production and insulation to defend body temperature, thus increasing rates of emaciation and so on until hypothermia and malnutrition overcame them.

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Poster Session – Sunday 1st July 2012

A13.41
Hypoxia prolongs digestion in the air-breathing striped snakehead fish *Channa striata*

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Here, we investigated whether an air-breathing fish, the striped snakehead *Channa striata*, is able to support increased metabolism during digestion when exposed to severe hypoxia. We measured the partitioning of oxygen uptake during digestion, and the duration and size of the postprandial metabolism (specific dynamic action), in hypoxic (30 mmHg) and normoxic (150 mmH) snakehead. The peak M_{O2} was significantly lower in hypoxic animals than normoxic animals, and the specific dynamic action response lasted six hours longer. The data show that despite *C. striata* being an air-breather, the limited aquatic respiration in severe hypoxia impairs its metabolic performance during digestion. Further studies are needed to investigate the underlying mechanisms for this.

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Poster Session – Sunday 1st July 2012

A13.42
Thyroid hormones as mediators of energy adjustments within ageing charadriiform birds

Kyle Elliott (University of Manitoba, Winnipeg, Canada), Vince Palace (Department of Fisheries and Oceans Canada, Winnipeg, Canada), John Speakman (University of Aberdeen, Scotland, UK) and Gary Anderson (University of Manitoba, Winnipeg, Canada)

Some studies have suggested a direct linkage between basal metabolic rate (BMR) and daily energy expenditure (DEE) because adjustments that maximize energy intake increase BMR. One possibility is that intrinsic modulators of BMR, such as thyroid hormones (T3 and T4), can impact both BMR and DEE, allowing animals to modulate energy expenditure across varying conditions such as ageing.

We tested these ideas in two species of seabirds (kittiwakes and murres) by measuring BMR, DEE and thyroid hormone levels. T3 and T4 were adjusted independently. Free T3 correlated with BMR but not DEE, showing that thyroid hormones are unlikely to be involved in linkages between BMR and DEE. T3 (and BMR) increased during stress following capture, as did the strength of the relationship between free and bound T3.

In contrast to long-lived birds with low energy costs (petrels), long-lived birds with high energy costs adjusted those costs with progressing age. We suggest that T3 plays a strong role in determining BMR and interactions between BMR and life history but does not play a strong role in determining DEE.

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Poster Session – Sunday 1st July 2012

A13.43
Autonomic influences on the cardiovascular system of the black and white tegu lizard

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There are no data regarding the cardiovascular autonomic control of the tegu lizard *Tupinambis merianae*, which presents a seasonal cycle of elevated activity (spring, summer) and reduced metabolism (winter). We investigated the autonomic influences on systemic arterial pressure (AP) and heart rate (HR), including the baroreflex regulation of AP, in unanesthetized tegus by determining AP and HR variations after peripheral injections of sodium nitroprusside (SNP), alpha and beta adrenergic agonists (phenylephrine and isoproterenol, respectively) and antagonists (prazosin and sotalol, respectively) and a cholinergic antagonist (atropine) during different seasons.

Basal HR, but not AP, was lower during winter than in the other seasons. All doses of SNP caused significant hypotensive and tachycardic responses during all seasons. These responses were less pronounced during winter. The tachycardic response to SNP was totally inhibited by double autonomic blockage (sotalol plus atropine). In contrast, phenylephrine induced a dose-dependent increase in AP but a weak bradicardic reflex during spring, summer and autumn. Smaller and dose-independent hypertensive and bradicardic responses to phenylephrine were observed during winter. Maximal and minimal HR responses were lower during winter but no significant seasonal effect on normalized baroreflex gain was observed. Regardless of season, prazosin did not change AP but inhibited the hypertensive effect of phenylephrine. Sotalol increased AP and inhibited tachycardia induced by isoproterenol.

Thus, *T. merianae* seems to present:

• a prominent baroreflex response to hypotension, but not to increased AP, during entire year; and

• beta adrenergic tone to vasculature, especially during summer–spring.

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Poster Session – Sunday 1st July 2012

A13.44

Levels of metallothionein gene expression in bank vole populations under metal exposure

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Organisms under stress conditions develop different mechanisms for coping with contaminants. One of these mechanisms is scavenging metal ions using metallothioneins. These cysteine-rich proteins are classified into four groups, among which two of them (Mt I and Mt II) are abundant in all tissues. The aim of our study was to assess metallothionein I gene expression in the liver tissues of the bank vole, *Clethrionomys (Myodes) glareolus*.

Animals were trapped in nine study sites in Poland (three clean mainland populations – Mikolajki, Niepołomice and Telesnica Oszwarowa; three clean islands – Dobskie, Dejguny and Skalista; and three polluted sites – Miasteczko Slaskie, Katowice and Olkusz). The polluted sites were located near zinc/lead smelters, where metal concentrations in animal tissues were significantly higher (up to 40 mg Cd/kg dry weight in the kidney) than in the tissues of animals from clean islands and open-mainland sites (up to 6 mg Cd/kg dry weight).

Gene expression was analysed using relative quantification. The lowest level of metallothionein I gene expression was noticed for Mikolajki. The highest level of expression was noted in the tissues of animals from polluted populations: expression was up to 7.5 fold higher than in Mikolajki. Those results show that metal exposure was high enough to activate detoxification mechanisms on gene expression levels.

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Poster Session – Sunday 1st July 2012

A13.45

Structural peculiarities of pronephros in Russian sturgeon, *Acipenser gueldenstaedtii* Brandt (Chondrostei)

Lyudmila S. Krayushkina (St. Petersburg State University, Russia), Arnold A. Gerasomov (St. Petersburg State University, Russia) and Andrey A. Kirsanov (St. Petersburg State University, Russia)

The morphology, histological and ultra-thin structures of the pronephros in the larvae of Russian sturgeon, *Acipenser gueldenstaedtii* Brandt, were investigated. The pronephros is the first kidney form during embryonic development in all vertebrates but the pronephros only remains in fish and amphibians after hatching and is the primary filtering and osmoregulatory organ in free-swimming larvae.

The pronephros of sturgeon larvae is presented by bilateral system of the pronephric tubules and the external glomus that is situated in closed pronephric chamber and is vascularized by dorsal aorta. On the each body side, the system starts from six short pronephric tubules. The posterior ends of these tubules connect with the collecting canal that passes into the excretory canal. The first pronephric tubule is opened into broadened part of the coelomic cavity. The anterior ends of the other five pronephric tubules open into the cavity of the pronephric chamber. Through the funnels the filtrate is drawn into tubules from chamber's cavity, as well as from coelom's cavity. The glomus of sturgeon has the same peculiarities of the thin structure that is typical and necessary for the function of a filtering organ: these are the capillaries with a fenestrated endothelium, the podocytes with foot processes (pedicles) that lean on the wall of the capillaries, and the mesanglial cells. The anatomic structure of the pronephros in acipenserids is significantly different from the structure of the pronephros in teleosts and has common principal peculiarities in common with the pronephros in amphibians.

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Poster Session – Sunday 1st July 2012

A13.46

Functional evolution of osmotic and ionic mechanisms in a number of acipenserids (Chondrostei)

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Acipenserids are freshwater fish by origin, but they presently inhabit water bodies of different salinities. A comparative investigation of the peculiarities of osmotic and ionic regulation in different ecological species of acipenserids during changes in medium salinity was carried out.

The immature specimens of seven species of acipenserids connected by varying degrees of sea conditions were used in our study. In a number of the species examined, the level of osmotic and ionic homeostasis depended on the salinity of the medium in which the different species primarily live. The higher the salinity of the medium; the higher the functional capacity of these mechanisms of homeostasis. These differences are attended by:

• changes in the function of the kidney (the increase of sodium reabsorption activity, the development of magnesium-secreting function and the development of the second proximal segment in the nephron);

• the intensification of sodium-reabsorption function of the intestine after the drinking sea water to retain water balance in the organism; and

• the intensification of the transport function of gill chloride cells, which is connected with changes in the ultra structure of these cells and with the increase of the Na^+/K^+-ATPase activity in these cells.

The data provide evidence of the functional development of the

mechanisms involved in osmotic and ionic homeostasis in a number of acipenserids and about the stimulating influence of environmental salinity on this process.

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Poster Session – Sunday 1st July 2012

A13.47

Leap of faith: Physiological drivers of emersion behaviour and its metabolic consequences in the oxyconforming galaxiid fish, *Galaxias maculatus*

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Near-coastal freshwaters in agriculturally-intense lowlands are increasingly prone to eutrophication, and consequently hypoxia. Navigating through these waters as part of an amphidromous lifecycle, the galaxiid fish inanga (*Galaxias maculatus*) needs to develop behavioural, physiological and/or metabolic strategies to deal with hypoxia exposure.

Behavioural experiments showed that inanga responded to hypoxia by increasing swimming activity, presumably in an attempt to avoid hypoxia. As hypoxia deepened, aquatic surface respiration was observed. Eventually, as environmental oxygen declined further, inanga emersed from the water and aerially respired. Emersed fish exhibited elevated oxygen uptake relative to fish that remained immersed in hypoxic waters. This was likely facilitated by the scaleless integument that appears to be a physiologically-viable surface in this species.

Studies examining the metabolic consequences of emersion *versus* remaining in aquatic hypoxia indicated that there were only minor changes between these two groups, suggesting little physiological advantage to emersion. Investigation of inanga oxygen consumption as a function of environmental oxygen levels demonstrated that inanga oxyconform, a very rare characteristic among fish, and one that might drive the emersion response.

In concert with data indicating that inanga have a relatively limited capacity for anaerobic metabolism, it is suggested that this species is poorly adapted to survive hypoxic waters in the long term. Our findings indicate that the increasing degradation of near-coastal waters might greatly impact the long-term sustainability of this economically-important and culturally-iconic fish species.

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Poster Session – Sunday 1st July 2012

A13.48

Salinity acclimation in an amphidromous fish (*Galaxias maculatus*) involves Na^+/K^+-ATPase isoform switching

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Euryhaline fish have to switch between active ion uptake and ion excretion in order to deal with the challenges posed by freshwater and seawater, respectively. This is especially true for inanga (*Galaxias maculatus*). This fish exhibits a specialized form of diadromy, known as amphidromy, which exposes it to both migratory- and tidal-related salinity changes.

Befitting their life history, we found that inanga successfully acclimated to salinities ranging from freshwater to 42‰. Direct transfer of inanga between waters of different salinity causes a 24-hour period of 'osmotic shock' where changes in plasma osmolarity and sodium levels were perturbed, but thereafter values adjusted to a new steady-state for the given salinity.

Molecular changes in the key ion transporter Na^+/K^+-ATPase (NKA) were also investigated. Sequencing determined the presence of multiple isoforms of the catalytic NKA α-subunit. Phylogenetic analysis showed that the inanga α-1a and α-1b formed a clade with the equivalent isoforms of rainbow trout, while another clade contained the α-1c isoforms of these species. The expression of all the inanga α-1 isoforms was modulated after seawater exposure, with α-1a and α-1c down-regulated following seawater exposure, and α-1b being up-regulated. Additional studies showed that these changes in isoform expression patterns conferred different biochemical properties (sensitivity to silver toxicity) in an *in vitro* NKA activity assay.

Our results indicate that inanga is an excellent osmoregulator. This ability is achieved by the rapid activation of physiological and molecular responses to salinity change.

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Poster Session – Sunday 1st July 2012

A13.49

Effects of gonadotropin-releasing hormone analogue treatment on ovarian activity in the animal model for polycystic ovary

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In the present study we evaluated the effects of gonadotropin-releasing hormone analogues (GnRH agonist and GnRH antagonist) treatment on ovarian activities in the animal model for polycystic ovary syndrome (PCOS). To develop the animal model for PCOS, prepubertal mice were injected daily (subcutaneously) with dehydroepiandrosterone (DHEA, 6 mg/100 g body mass) for 30 days (PCO-mice). These mice developed numerous cystic follicles showing morphological features of polycystic ovaries, with increased body weight, increased circulating testosterone, decreased oestradiol and anovulation. The PCO-mice were then treated daily with either GnRH agonist (GnRH-Ag; 5 or 25 µg) or GnRH antagonist (GnRH-Anta; 5 µg or 25 µg) for eight days and their effects on follicular development, steroidogenesis, luteinisation and apoptosis were investigated.

PCO-mice treated with GnRH-Ag and GnRH-Anta suppressed the formation of cystic follicles and induced ovulation, as confirmed by the presence of corpus luteum, decreased circulating testosterone and increased oestradiol levels. The mice treated with GnRH-Ag showed some haemorrhagic follicles, suggesting increased luteinising hormone hyperstimulation with increased ovarian expression of luteinising hormone receptor, 3-beta–hydroxysteroid dehydrogenase and steroid receptor proteins. Increased expression of these proteins might be correlated with active follicular development, ovulation and hormonal luteinisation. Mice treated with GnRH-Anta, however, showed corpus luteum containing hypertrophied and vacuolated luteal cells, decreased ovarian expression of luteinising hormone receptor, 3-beta–hydroxysteroid dehydrogenase and steroid receptors.

This study therefore suggests that treatmenting the PCO mice with GnRH-Ag suppressed the development of cystic follicles and induced ovulation by decreasing the androgen level. Thus, treatment with GnRH-Ag reverses the PCO-like features.

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Poster Session – Sunday 1st July 2012

A13.50
Daily energy expenditure of adult green turtles (*Chelonia mydas*) during foraging and nesting migration

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Knowledge of the energy requirements of marine turtles is fundamental for our understanding of their physiology, ecology, and ultimately population dynamics, yet little is known about their energetic requirements during the different stages of their oceanic life. We combined the accelerometry technique and a recent accelerometry-derived energetics model to investigate the daily energy expenditure (DEE) of adult green turtles during their foraging phase on a seagrass meadow (Mayotte Island) and during their breeding season (Moheli Island).

At the foraging site, 10 turtles were equipped throughout the year with a data logger recording depth, water temperature (T_w), and two-axis acceleration for up to five days. In Moheli, a breeding female was captured at the nesting beach, equipped and released ~150 km southwest of Moheli, from where she swam back to the nesting beach within four days.

Throughout the year, foraging turtles were feeding at shallow depth (1–5 m) during daytime and resting at greater depth (10–15 m) at night. DEE was low at 9.9 ±1.1 kJ kg^{-1} d^{-1} during the summer (T_w: 28.9 ±0.0° C) and decreased further by ~14% during the winter (8.5 ±0.8 kJ kg^{-1} d^{-1}; T_w: 25.7 ±0.4°C). These values correspond to 1.3 and 1.5 times their resting rates during summer and winter, respectively. By contrast, the translocated turtle swam almost continuously, remaining at a shallow depth during the day and moving to greater depth (~20 m) at night. Accordingly, DEE during this four-day-period averaged 16.7 ±1.4 kJ kg^{-1} d^{-1} (T_w: 25.9 ±0.2°C), which amounts to 2.9 times the respective resting rate.

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Poster Session – Sunday 1st July 2012

A13.51
Heat loss and the energetic costs of shallow and deep diving in double-crested cormorants (*Phalacrocorax auritus*)

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Avian divers are confronted with a number of physiological challenges when foraging in cold water, especially at depth. Cold water temperatures and a reduction in body insulation, due to the increase in pressure with dive depth, will elevate the energetic costs of foraging in these endotherm divers. This could be especially severe in cormorants, where a partially-wettable plumage might greatly increase heat loss at depth.

Dive costs of double-crested cormorants diving vertically to 10 m were shown to be substantially greater (~22%) than when birds swam within a horizontal tank at 1 m depth. This difference was attributed to the supposedly increased heat loss at greater depth, elevating thermoregulatory costs.

Heat flux has not been measured directly in freely diving birds. From temperature recordings during diving, heat conservation mechanisms, such as peripheral vasoconstriction, have been indicated for a number of avian divers. We used heat flux sensors attached to various sites on the plumage of double-crested cormorants to measure heat loss during dives to 3 and 10 m. Our results show that heat loss varied greatly with location, in accordance with plumage thickness, but was considerably less than previous estimates based on a physical model. Peak heat loss at all sites was reached before final depth and was significantly greater during deeper dives, but mean heat loss did not differ with dive depth. Elevated dive costs during deep vertical dives, when compared with shallow horizontal dives, are therefore more likely related to an increased locomotor effort, rather than thermoregulatory costs.

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Poster Session – Sunday 1st July 2012

A13.52
Does trophic contamination by polycyclic aromatic hydrocarbons impair metabolic and swimming performance in zebrafish (*Danio rerio*)?

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Swimming and metabolic performance are variables that enable us to access the effects of environmental conditions on fitness in aquatic species, such as fish. The aim of our study was therefore to measure the effects of pyrolytic polycyclic aromatic hydrocarbons (PAH) on the critical swimming speed (U_{crit}), the associated energetic costs and the aerobic metabolic scope in zebrafish *Danio rerio*.

D. rerio were contaminated via the trophic pathway with environmentally-relevant PAH mixtures at several sub-lethal concentrations. Larvae were fed from the first meal (four days post fertilization) onward with contaminated dry and size-adapted pellets. Juveniles (70 days post fertilization were individually challenged following a step protocol in a 170 ml-swim tunnel, where they were forced to swim until fatigue. Concomitantly, oxygen consumption was measured at each 20 minute-long step that allowed the assessment of the active metabolic rate and the standard metabolic rate. Aerobic metabolic scope was then calculated giving insights into the metabolic performance of *D. rerio*.

No significant difference was observed whatever the concentration of PAH in diet, which suggests that the locomotor performances tested and the associated energetic costs were not impaired by PAH. Future results on adults and larvae completed by experiments regarding escape performance and cardiac development will permit us to improve our understanding of the potential effects of PAH on the physiology of *Danio rerio*.

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Poster Session – Sunday 1st July 2012

A13.53
Ballistic food transport in frugivorous Paleognathous and Neognathous birds

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The basic mechanism of food transport in tetrapods is lingual-based. Neognathous birds use this mechanism for exploiting a large diversity of food resources, whereas paleognathous birds use cranio-inertial

mechanism with or without tongue involvement.

We compared food transport mechanism in Paleognathous birds (rhea [*Rhea americana*] and cassowary [*Casuarius casuarius*]) and Neognathous birds (toucan [*Ramphastos toco*] and hornbills [*Aceros* sp.]). In spite of different positioning of the head at the beginning of the transport phase, i.e. horizontal in *C. casuarius* and vertical in *R. americana*, the capture and transport of food items are similar. Only one transport cycle is used for throwing the food from the tip of the beak to the pharynx. The food is projected by a rotational backward movement of the head in neognathous birds and in cassowary. In rhea, the head displaces through a rather vertical backward–forward movement, the beak opens suddenly and the food continues its movement before being swallowed by a movement of the head in the opposite direction. In both species, the food follows a ballistic curve. The food enters in the pharynx at the end of its parabolic trajectory in toucan, hornbill and cassowary, whereas in rhea the transport cycle is shorter and the food enters the pharynx at the 'ascendant' stage of the ballistic curve.

In conclusion, a similar mechanism of ballistic transport is used by paleognathous and neognathous birds, the differences observed depending on the initial position of the head and dynamic characteristics (velocity) of the food.

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Poster Session – Sunday 1st July 2012

A13.54

Evaluation of *in vitro* cytotoxicity of an ethanolic extract of *Bacopa monnieri* L. in the MCF 7 and SiHa cell lines

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The ethanolic extract from *Bacopa monnieri* is a rich source of different antioxidant and free radical-scavenging secondary plant metabolites such as bacoside A and B, Brahmin, cucurbitacins and apocynin. The cucurbitacins and apocynin also have strong anti-tumorigenic and anti-proliferative activities.

The present study was therefore carried to evaluate the *in vitro* cytotoxic activity of *Bacopa monnieri* ethanolic extract on MCF7 and SiHa cell lines using the trypan blue dye exclusion method and cytotoxicity assay up to 72 hours after exposure at intervals of 24 hours. The trypan blue dye bound to the mitochondria of viable cells, showing the formation of formazon crystals. The absorbance of these crystals was measured using a microplate reader at a wavelength of 570 nm with a reference wavelength of 630 nm. The results of the assay extract showed good anti-cancer activity.

The extract was further fractionated to isolate the lead molecule. Testing of the anticancer potentials of different fractions from this study is in progress in our laboratory.

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Poster Session – Sunday 1st July 2012

A13.55

Acute responses of brown mussel (*Perna perna*) exposed to sub-lethal copper levels: Integration of physiological and cellular responses

Andre Vosloo (University of KwaZulu-Natal, South Africa), Joseph Sara (University of Limpopo, South Africa) and Dalene Vosloo (University of KwaZulu-Natal, South Africa)

This study examined the effect of sub-lethal copper levels on selected physiological and cellular responses of the marine bivalve *Perna perna*. Animals were exposed to five environmentally-relevant copper concentrations of 12.5, 25.0, 37.5 and 50.0 µg L^{-1}. Metal accumulation was found to be significantly increased at the two higher copper concentrations after 24 hours of exposure.

Physiological responses found to increase during acute copper exposure included mucus secretion rate (at 25 and 50 µg L^{-1} copper), and nitrogen excretion rates and oxygen consumption rates (both at 25 and 50 µg L^{-1} copper).

Perna perna changed its substrate utilization at 25, 37.5 and 50 µg L^{-1} copper in favour of protein-based metabolism. A higher degree of reactive oxygen species-induced DNA damage was observed at acute exposure to 37.5 and 50 µg L^{-1} copper. The filtration rate was unchanged during acute copper exposure.

A model is proposed that integrates cellular and physiological responses to copper during short-term acute and long-term chronic exposures.

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Poster Session – Sunday 1st July 2012

A13.56

Death is not the end: Postmortal changes of rat skeletal muscle during the rigor mortis period

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After death, a stiffness of muscle tissue evolves that is referred to as rigor mortis and is mediated by the decrease in ATP storage and a concomitant increase in intracellular Ca^{2+}. This state of constant contraction remains until structural proteins are broken by proteolysis, like the costameric protein desmin, for example, which is degraded within the early postmortal hours (O'Halloran *et al*., 1997; Veiseth *et al*., 2004). The rigor mortis period therefore seems to be a promising time span for analysing the postmortal changes in muscle tissue.

Although a fine structural analysis of postmortal rat skeletal muscle was carried out by Collan and Salmenperä (1976), no emphasis was placed on the rigor mortis period. For light and electron microscope analyses in this study, an adult rat was sacrificed and every hour from zero to 16 hours post-mortem two samples were dissected from its thigh muscles at room temperature. One of these samples was physically fixed and cut on a cryostat for analyses of enzymatic activities and the other one was further processed for electron microscopy.

Although the activity of the muscle proteins (LDH, SDH, mATPase and AChE) analysed remained stable throughout the rigor mortis period, a variety of intracellular alterations was observed on electron micrographs. These included a dilatation of certain intracellular organelles, an increase of condensed chromatin in the nucleus and modifications of membranous structures. As expected, most of these changes arose at the onset of rigor mortis resolution, shown to set in at seven hours post-mortem by rigor tension measurements.

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Poster Session – Sunday 1st July 2012

A13.57
Fluctuating thermal and hydration regimes in a chill-susceptible insect

Leigh Boardman (Stellenbosch University, South Africa), Jesper G. Sørensen (Aarhus University, Denmark) and John S. Terblanche (Stellenbosch University, South Africa)

Fluctuating thermal regimes (FTRs) consisting of longer-term cold exposure broken by repeated short warm temperature fluctuations have been shown to improve survival and fecundity in a variety of insects. Theoretically, the warming periods allow for repair of any damage that has occurred at the lower temperatures, thereby acting as a protective mechanism. By contrast, fluctuating dehydration–rehydration regimes (FHRs) have been less comprehensively researched.

Here, we used chill-susceptible final instar larvae of the false codling moth (*Thaumatotibia leucotreta*) as a model organism for investigating whether the effects of controlled FTRs and FHRs are protective or accumulate damage at the cellular and whole-organism levels. Baseline experiments that investigated water loss rates, time to death under either starvation or desiccation, and plasticity of thermal tolerance were also conducted in order to better characterize the study organisms' thermal biology in relation to fluctuating stress. At five time points during each experiment of FTR, FHR and the relevant controls, metabolic rate was recorded, while body water and lipid content, heat shock protein 70 expression levels and mortality were assayed.

The data showed that both FTR and FHR significantly affect body water content but not lipid content, over the course of the experiment. Preliminary analysis suggests that *T. leucotreta* do not rely on heat shock protein 70 to survive FTR or FHR, possibly indicating that the experimental conditions were not stressful. Alternatively, other mechanisms might act to reduce the impacts of the fluctuating stress. These preliminary results are in general agreement with other literature published to date.

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Poster Session – Sunday 1st July 2012

A13.58
Benthic fauna response to coastal hypoxia and anoxia: An integrated, community-level *in situ* approach

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No other environmental parameter worldwide has changed as severely as dissolved oxygen (DO). Presently, nearly 400 so-called 'dead zones' have been identified in nearshore areas around the globe, impacting marine communities from the molecular to the ecosystem level. The Northern Adriatic Sea (Mediterranean) serves as a model for repeated seasonal low DO events including benthic mortalities. Often the onset and extent of such collapses are difficult to predict, hindering full documentation in the field.

Using Plexiglas chambers, we experimentally induced small-scale anoxia from one week to one year at 24 m depth to mimic the forcing conditions during large-scale oxygen deficiency events. The sediment geochemistry was examined using different techniques, and meiofaunal (harpacticoid copepods and foraminiferans) density and diversity were analysed after each experiment. The impact on macrofauna (i.e. behavioural responses, intra- and interspecific interactions and mortality sequences) was documented with a time-lapse camera.

Short-term anoxia had only a minor impact on benthic foraminiferans. After two months, however, diversity had decreased markedly and the fauna was strongly dominated by *Eggerella scabra*. The copepods responded rapidly to hypoxia, and density even dropped to zero in anoxia (most tolerant: family Cletodidae). While hypoxia affected individual macrofauna abundance, anoxia significantly reduced species diversity.

This approach yields a comprehensive picture of events during and after anoxia by tackling individual to community-level responses from different perspectives in the natural environment. The results provide information on the sensitivity and function of the benthic compartment and help interpret post-disturbance community composition and recovery potential.

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Poster Session – Sunday 1st July 2012

A13.59
Cardioventilatory and stress physiological responses to electric field exposure in Arctic char, *Salvelinus alpinus* L.

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Electric field exposure is used to stun or immobilize fish prior to slaughter in the aquaculture industry and for field sampling purposes (i.e. electrofishing), but the physiological response of fish to this exposure is incompletely understood. Here we report on changes in blood pressure, heart and ventilation rates and haematological variables in chronically cannulated Arctic char (*Salvelinus alpinus*) in response to exposure to an electric field of 4 V/cm (125 Hz) for 5 and 30 s.

Both durations of exposure resulted in a brief (total duration: 5.2 and 6.0 s, respectively) four-fold blood pressure increase above resting levels.

The 5 s exposure was followed by a period of cardiac and ventilatory arrest (for 35 and 176 s on average, respectively), but cardioventilatory activity recovered in 10 out of 11 fish. Despite this, signs of systemic stress responses were evident after the exposure. These included moderate hypertension, increased ventilation amplitude, increased plasma cortisol levels and altered hydromineral balance.

After the 30 s exposure, cardiac activity initially appeared to recover, but subsequently declined. Ventilation did not recover. It is suggested that circulatory failure is due to cardiac ischaemia resulting from ventilatory failure rather than from instantaneous and irrecoverable cardiac arrest from the electric field exposure *per se*, in fish that fail to recover from exposure to an electric field in water.

The brief dramatic hypertension observed in char might partly explain the haemorrhages that are frequently observed in the electrically stunned fish of some species.

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Poster Session – Sunday 1st July 2012

A13.60
The role of Hif 1α in the paired, bipectinate gills and foot muscle of South African abalone (*Haliotis midae*) during hypoxia

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The genus *Haliotis* is a primitive vetigastropod and has retained both gills. Previous research has found that one gill supports basal metabolism, while the other gill is recruited during periods of increased oxygen demand. This genus has also been found to exhibit a reverse Bohr shift to facilitate loading of haemocyanin in the gills. As hif-1α is known to initiate a cascade of events to improve the oxygenation of tissue during periods of hypoxia, and might therefore drive the recruitment of the left gill, the current study was designed to assess whether hif-1α proteins accumulate in the gills of *H. midae* during the first hours of hypoxia. We also aimed to establish whether the oxygen loading of haemolymph changes during short-term hypoxia events. Our results indicate a subtle but variable change in hif-1α levels in the gills, with improved oxygen loading of haemolymph when animals are exposed to emersion for six hours. This study will enable us to further understand the effect hypoxia has on aquaculture and the natural distribution of this species.

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Poster Session – Sunday 1st July 2012

A13.61
Temperature modulates fibre numbers in zebrafish muscle hyperplasia but not in initial myogenesis

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Muscle fibre number is a prominent measure of muscle growth modulation by thermal influence in teleost fish. This is particularly important as the final fibre number is a key determinant of ultimate body size. It has been shown that the final number of muscle fibres in the zebrafish, *Danio rerio,* can be influenced by temperature treatment. Neither the cellular growth mechanisms that regulate fibre generation nor the life stages that are crucial to this process have yet been determined in detail.

To further elucidate these two aspects, zebrafish were reared and maintained at three different temperatures (25.0, 28.5 and 31.0°C). Samples were taken from hatching to adult stage. Digital morphometry was employed to assess muscle cellularity in distinct myotomal zones, allowing detailed insight into cellularity change and thermal influence on muscle growth dynamics.

We show that temperature exerts no influence on fibre generation during the initial phase of myogenesis, resulting in similar fast fibre numbers at hatching in all temperature groups. Clear differences are found, however, in fish from the midlarval stage onwards. They are most prominent at the larval–juvenile transition and can still be detected in almost fully grown, sexually mature adults five months post fertilization. Cellularity analysis reveals that the differences in fast fibre numbers are due to thermal modulation of both the onset and the intensity of the two hyperplastic growth mechanisms, stratified and mosaic growth.

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Poster Session – Sunday 1st July 2012

A13.62
Measuring the impact of transport on the stress response of ornamental marine fish

David C.C. Wolfenden (University of Chester, UK), Tom G. Pottinger (Centre for Ecology and Hydrology, UK) and Lynne U. Sneddon (University of Chester, UK)

The transport of marine ornamental fish involves potential stressors; these include capture, handling, confinement and changes in water quality. There have been few attempts to evaluate the effects of transport in the ornamental fish trade. Simulated transport to industry standards was conducted using common clownfish (*Amphiprion ocellaris*) to determine the extent to which this process results in a stress response by measuring non-invasive behavioural and physiological parameters.

Fish underwent simulated transport for 30 minutes, two hours, 24 hours, 48 hours and 72 hours, while a control group experienced capture and handling alone. Water samples taken from the transport container were analysed to determine excreted cortisol concentrations as well as water quality indicators (NH_3/NH_4^+; NO_2^-; NO_3^-; pH; and alkalinity). Subsequent behavioural measurements were recorded following simulated transport to determine recovery.

Fish responded to initial capture, restraint and transport with a marked increase in cortisol; after a period of 24 hours fish appeared to adapt, with a decline in the rate of cortisol production. Behavioural responses demonstrated that latency to feed was greatest in fish experiencing longer transport, suggesting that behaviour is impaired by transport for longer than 24 hours. Water quality did not have a significant effect on the stress response since fish that had improved water quality during longer transport periods still had elevated cortisol production.

The results demonstrate that the transport procedure was stressful and might present considerable welfare issues.

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Poster Session – Sunday 1st July 2012

A13.63
Aerobic metabolism in the killifish *Nothobranchius furzeri*, a vertebrate with an extremely short life span

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Nothobranchius furzeri is an annual fish with an extremely rapid lifecycle of three to six months. It is therefore considered a good vertebrate model for ageing studies. Even if the short lifespan confers specific adaptations, however, the physiology of this species is not well known. The objective of our study was therefore to learn more about the bioenergetics of this vertebrate model. In order to determinate standard and maximal metabolic rates, oxygen consumption was measured by intermittent flow respirometry. Adults of approximately three months of age were individually tested in respirometer chambers (diameter 7.5 cm, volume 0.179 L) over two days. Oxygen consumption was continuously recorded in two main activity statuses: individuals were stressed by chasing them until fatigue to estimate the maximal metabolic rate; and then they were left undisturbed to measure standard metabolic rate.

Results will be discussed through a comparison between the metabolism of this short lifespan species and other Nothobranchiidae.

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Poster Session – Sunday 1st July 2012

A13.64
Thermal limitations of aerobic scope and cardiac capacity in summer acclimated perch (*Perca fluviatilis*)

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Recent studies have indicated that predicted increases in temperature of only a few degrees can affect the performance of aquatic ectothermic animals to a degree that will potentially impact aquatic ecosystems and commercial fisheries. In fishes it has been proposed that upper thermal tolerance limits are set by the cardiorespiratory system's ability to transport oxygen so that heat stress coincides with dwindling aerobic scope at high temperatures. The aim of the present study is to characterize the interaction between oxygen transport capacity and temperature tolerance of the European perch (*Perca fluviatilis*). Specifically, the relationship between cardiac performance and thermal tolerance was studied by measuring aerobic scope, temperature tolerance, heart rate and force development of cardiac preparations at different temperatures (5–27° C) in fish acclimated to summer conditions. As expected, resting metabolic rate increased in a near-exponential manner while maximal oxygen transport capacity increased almost linearly in the same temperature interval. These different patterns resulted in an absolute maximal aerobic scope a 21°C, while the maximal factorial aerobic scope was found at 10° C.

We are currently characterizing the cardiac capacity through *in vivo* recordings of maximal and resting heart rate as well as in *in vitro* cardiac preparations exposed to different temperatures and oxygen levels. Using these results, we will to correlate our findings with *in vivo* oxygen transport capacity and thermal tolerance and discuss this in relation to the putative constraints of the cardiovascular system.

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Poster Session – Sunday 1st July 2012

A13.65
The role of gastric digestion on specific dynamic action and postprandial organ growth in pythons (*Python regius*)

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Digestion is associated with increased oxygen consumption in animals (specific dynamic action), but the specific contributions to the metabolic rise remain unclear. The importance of gastric acid secretion and gut hypertrophy has been emphasized in previous studies. Thus, gastric digestion has been reported to account for up to 55% of the specific dynamic action response. To investigate the role of gastric processes, we blocked the pyloric sphincter in snakes to exclude any intestinal contribution to digestive processes and let the snakes eat voluntarily.

Animals subjected to a pyloric block showed lower oxygen consumption compared to controls that had not been operated on (626 ±38 and 1,598 ±88 ml O_2 kg^{-1}). Animals given an amino acid solution (peptone) directly into the intestine (gastric bypass) showed specific dynamic action responses similar to animals fed the same solution into the stomach, thus experiencing complete digestion (1,078 ±80 and 1,244 ±85 ml O_2 kg^{-1}). Animals that had not been operated on (controls) showed significant postprandial organ growth of the liver, stomach, pancreas and small intestine. No organ growth was found in the pyloric block or gastric bypass groups.

These results indicate a much lower gastric contribution to the specific dynamic action response than previously reported. They also indicate that organ growth is not initiated in the stomach or as a response to protein.

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Poster Session – Sunday 1st July 2012

A13.66
Development of unpaired fin muscle in teleost fish

Christina Kreutzer (University of Salzburg, Austria), Peter Steinbacher (University of Salzburg, Austria) and Walter Stoiber (University of Salzburg, Austria)

The cellular processes that generate the unpaired fin muscles of teleost fish are of great importance because they might help to resolve the question of how these fins arose during vertebrate evolution. While the development of paired fin muscles has recently been characterized, almost nothing is known about the equivalents in unpaired fins.

The present work investigates the development of the supracarinalis muscles during early development of the European pearlfish *Rutilus meidingeri*, Cyprinidae. Immunostaining techniques are combined with classical histology to trace the origins of these muscles, which constitute longitudinal strands to either side of the dorsal midline. These strands span from the dorsal fin anteriorly towards the head and posteriorly towards the tail fin.

Results to date demonstrate that supracarinalis muscle formation commences in the anterior trunk in the early larval period. At this developmental stage, mitotically-active Pax7+ muscle precursor cells emerging within the mesenchyme area between the dorsal apices of the myotomes give rise to unsegmented muscle strands that initially only consist of slow fibres. At the position of the dorsal fin, these strands attach via tendons to the more diversified system of fin ray muscles (erectors, depressors and inclinators) that embrace the radials of the fin.

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Poster Session – Sunday 1st July 2012

A13.67
Novel approach to delimitate the time since death by means of skeletal muscle tissue

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Skeletal muscle tissue of two pigs stored at 22ºC for five days or 4ºC for 21 days was used to approach a central topic in forensic research, namely the delimitation of the time since death (DTSD). Light and electron microscopic as well as protein analyses were performed. For comparative purposes, 12 selected human corpses were sampled to demonstrate whether porcine skeletal muscle is feasible as forensic model tissue and a skeletal muscle-based approach is applicable for DTSD.

Although gross histological changes of connective and muscle tissue were obtained, light microscopy seems to be rather inaccurate compared to the fine structural method. Due to the specific post mortem time-dependent appearance of various intracellular organelles, a proper DTSD might be possible. The third methodological protein approach seems to be the most promising, showing short-term stabilities for a minority of proteins, while the majority of investigated proteins displayed characteristics as long-term estimators for DTSD. Due to specific patterns and the possibility of determining definite constraints of presence, absence or pattern alterations of single proteins, this protein-based approach for DTSD has a high potential for routine application in daily forensic medicine.

In conclusion, this study underlines the potential of skeletal muscle as forensic model tissue, especially with respect to later post mortem phases, which so far lack feasible methods to delimitate the time since death.

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Poster Session – Sunday 1st July 2012

A13.68
Triploidy impairs hypoxia tolerance in four strains of rainbow trout (*Oncorhynchus mykiss*)

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The goal of this project was to determine why triploid (3n) rainbow trout exhibit higher mortalities in the wild compared with their diploid (2n) counterparts. To accomplish this goal, we measured a number of physiological performance (e.g. swimming performance and routine metabolic rates) and environmental tolerance (e.g. hypoxia and thermal tolerance) parameters in three wild strains and one domesticated strain of juvenile rainbow trout raised as both 2n and 3n. The three wild strains used in this study differ in life history characteristics (e.g. river *versus* lake dwelling with varying levels of predation, competition and productivity).

We found significant effects of strain on all performance and tolerance parameters measured, except thermal tolerance. The effect of triploidy on performance and tolerance was only significant in our assessments of hypoxia tolerance, where 3n fish of all strains were consistently more sensitive to hypoxia than their 2n counterparts. The effects of triploidy on hypoxia tolerance were repeatable over two separate brood stocks. In an attempt to explain the effects of triploidy on hypoxia tolerance, we assessed critical oxygen tensions in the 2n and 3n versions of our four strains of trout, but there was no relationship observed between hypoxia tolerance and critical oxygen tensions.

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Poster Session – Sunday 1st July 2012

A13.69
Hypoxia tolerance of *Danios* and *Devarios*

Lili Yao (University of British Columbia [UBC], British Columbia, Canada) and Jeffrey G. Richards (UBC, British Columbia, Canada)

There is a long-standing hypothesis in the literature that fish with a higher capacity for anaerobic energy production will be more hypoxia tolerant than fish with a lower capacity but this relationship has not been thoroughly studied. This project aims to examine this relationship using the phylogenetically-corrected comparative approach on zebrafish and its close relatives (other *Danios* and *Devarios species*).

Hypoxia tolerance was assessed by measuring critical oxygen tension (P_{crit}), time to lose equilibrium at 12 torr, and the oxygen tension that yielded loss of equilibrium of 50% of fish over eight ours (EC_{50}). Critical oxygen tensions were similar among all 10 species examined, but loss of equilibrium and EC_{50} varied among species and showed the same relationship. The most hypoxia-tolerant species was *Danio kyathit* and the most hypoxia-sensitive species was *Danio choprai*. The capacity for anaerobic energy production was investigated by assessing metabolite concentrations (glycogen, glucose, creatine phosphate and ATP) and enzyme activities (pyruvate kinase, lactate dehydrogenase and creatine phosphokinase) in muscle, liver and brain from normoxia-acclimated fish.

The expected correlations between hypoxia tolerance and metabolite concentrations or enzyme activities were not found.

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Poster Session – Sunday 1st July 2012

A13.70
Low temperature delays demographic ageing more than it slows the rate of ageing: Experimental study in the blowfly *Calliphora stygia*

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Low temperature extends the lifespan of ectotherms. Early studies used average lifespan to determine the influence of temperature on the rate of ageing. A more sophisticated analysis of the rate of ageing is to measure the change in age-specific mortality rate of a population.

We studied the influence of temperature on ageing in the blowfly (*Calliphora stygia*) by measuring egg-laying, food consumption and age-specific mortality in replicate populations at 12, 15, 20, 25, 29 and 35ºC. This covered the species' physiological temperature range (egg-laying occurred at all but the two extreme temperatures). Daily food consumption significantly increased from 12–20ºC but not from 20–35ºC, suggesting that temperature effects are not rate-of-living effects. Demographic analysis showed that at 29ºC and 35ºC, adult mortality could be described by a single Gompertzian equation (indicating demographic senescence). At 12–25ºC, the period of demographic senescence was preceded by a period of minimal change in age-specific mortality (i.e. negligible demographic senescence). This period of negligible senescence varied from 72 days at 12ºC to 39 days at 25ºC and showed a greater sensitivity to temperature than the slope of the later Gompertzian relationship (i.e. the rate of ageing). Temperature crossover experiments (between 15ºC and 29ºC) confirmed the conclusion that lowered temperature delays 'senescence' more than it slows the demographic rate of ageing. Cellular damage was measured as fluorescent age-related glycation end-products (AGE pigments). At all temperatures, AGE pigment increased with age. The rate of AGE pigment accumulation was slower at low temperatures.

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Poster Session – Sunday 1st July 2012

A13.71
Occurrence of rodlet cells and eosinophilic granule cells in common carp (*Cyprinus carpio* L., 1758) reared in a semi-intensive system

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Common carp is one of the most important species in freshwater aquaculture. The dominant system of carp rearing is semi-intensive in earthen ponds. Some of the food is provided from the pond ecosystem during zooplankton and bottom fauna abundance, while different feed is added when natural food is depressed. Due to seasonal changes and technological processes applied during the production cycle, this type of aquaculture comprises changes in environmental conditions that contribute to the presence of different parasites.

A field study was conducted in which histopathological screening of the hepatopancreas, gills and intestine of farmed carp was carried out in order to assess the presence of parasites. The tissues were stained with hematoxylin and eosin, and Alcian blue-periodic acid-Shiff staining. The most common parasites observed were *Trichodina* sp., sometimes found in large numbers on gills. In the liver, the occurrence of nematode

larvae was significant. There were no differences between the numbers of parasites found in ponds where different added feeds were used (cereals, pelleted and extruded).

The presence of rodlet and eosinophilic granule cells (EGCs) was confirmed in hepatopancreas in the vicinity of nematode larvae. The majority of rodlet cells were noted in bile duct epithelium and the pancreas. The rodlet cells were often accompanied with EGCs, while EGCs were also observed in large numbers near parasite larvae. EGCs were frequently degranulated, releasing mediators of inflammation near parasites. These findings confirm the inflammatory character of EGCs and rodlet cells, their role still remaining an area of controversy.

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Poster Session – Sunday 1st July 2012

A13.72

Calorie restriction restores antioxidant capacity and prevents age-related changes in catecholamine biosynthesis in the hypothalamus

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Ageing is associated with hypertension and an increase in sympathetic nervous system (SNS) activity that is characterized by an increase in catecholaminergic enzymes in the adrenal medulla and by a reduction in antioxidant capacity in the hypothalamus. Caloric restriction has been shown to alter catecholaminergic enzymes in the periphery and increase the level of antioxidants in the brain. We hypothesized that 40% caloric restriction would prevent age-related changes in tyrosine hydroxylase in the adrenal medulla and hypothalamus and reverse the age-related depletion of hypothalamic antioxidants.

Young (eight-month-old) and old (29-month-old) male F344 × BN rats were fed ad libitum or were 40% calorie restricted. With age, there was a 60% increase in tyrosine hydroxylase (TH) in the adrenal medulla. Caloric restriction did not alter adrenal TH in young, but significantly decreased adrenal TH 67% (p=0.04) in old rats. TH decreased by 14% in the hypothalamus with age. This decrease was prevented by caloric restriction. Finally, caloric restriction increased central antioxidant capacity in aged rats, including a 52% (p=0.00) increase in manganese superoxide dismutase and a 57% (p=0.00) increase in catalase.

These data indicate that 40% caloric restriction reverses the age-related changes in TH in the adrenal medulla and the hypothalamus. Moreover, 40% caloric restriction restores the age-related decline in hypothalamic antioxidant capacity. Collectively, these changes might play important roles in attenuating the age-related increase in sympathetic nervous system activity. Importantly, these results suggest that calorie restriction could be a potential therapeutic strategy to attenuate increased sympathetic nervous system activity and hypertension in the elderly.

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Poster Session – Sunday 1st July 2012

A13.73

Exceptional cardiac anoxia tolerance in tilapia (*Oreochromis* hybrid)

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Anoxic survival requires the matching of cardiac ATP supply (i.e. maximum glycolytic potential, MGP) and demand (i.e. cardiac power output, PO). We examined the hypothesis that the hypoxic cardiac down-regulation previously observed *in vivo* in tilapia (*Oreochromis* hybrid) represents a physiological strategy to reduce routine PO to within the heart's MGP.

We developed an *in situ* perfused heart preparation for tilapia (*Oreochromis* hybrid), and characterized routine and maximum cardiac performance at 22ºC under normoxic (>20 kPa O_2) and severely hypoxic perfusion conditions (<0.20 kPa O_2 + pH 7.75 + pH 7.25 [acidosis] or + 1 mM NaCN [chemical anoxia]). Contrary to the cardiac down-regulation previously observed *in vivo*, routine cardiac performance *in situ* was maintained under all severely hypoxic conditions. We conclude that tilapia do not require *in vivo* down-regulation of routine cardiac performance in hypoxia to balance cardiac energy supply and demand. Indeed, the MGP of the tilapia heart was exceptional (172 nmol ATP s^{-1} g^{-1}, sustaining an anoxic pO_{max} of at least ~3.1 mW g^{-1} ; 22ºC), and substantially greater than that previously estimated for ectothermic vertebrate hearts (~70 nmol ATP s^{-1} g^{-1}, sustaining a pO_{max} of ~0.7 mW g^{-1}; 15ºC). The additional challenge of acidosis during severe hypoxia decreased the pO_{max} by 30%, suggesting potential direct effects of acidosis on cardiac contractility. We conclude that the exceptional MGP and anoxic performance of the tilapia heart conveys a previously-unreported anoxia tolerance, but a tolerance possibly tempered *in vivo* by acidotic waste accumulation during anoxia.

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Poster Session – Sunday 1st July 2012

A13.74

Effects of endurance training on humans with a single nucleotide polymorphism in the PGC1α gene

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PGC1α (peroxisome proliferator–activated receptor γ co-activator 1α) is an important regulator of mitochondrial biogenesis and a master regulator of enzymes involved in oxidative phosphorylation. Recently, the single nucleotide polymorphism (SNP) Gly482Ser in the PGC1α gene has been shown to affect insulin sensitivity, blood lipids and muscle parameters such as myofibrillar structure and mitochondrial oxidative capacity. Individuals that carry this SNP have a reduced physical fitness and a higher risk of type 2 diabetes.

Following the hypothesis that such persons should have reduced exercise trainability, we investigated the responses of sedentary men with the Gly482Ser SNP and a control group to endurance training (cycling, 3 × 60 minutes/week, 10 weeks, HR@70–90% V_{O2peak}).

Results show that persons carrying this SNP had a diminished exercise effect at the sub-maximal performance level compared to the control group. Preliminary quantitative data further demonstrate that capillary supply, mitochondrial density and content of slow contracting oxidative fibres in the vastus lateralis muscle increased significantly after training in both the SNP group and the control group.

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Poster Session – Sunday 1st July 2012

A13.75

Nitric oxide and the plasticity of cardiac performance to temperature changes in the teleost *Anguilla anguilla*

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The intrinsic regulation of the heart, i.e. the Frank-Starling law, is a fundamental property of the vertebrate myocardium that means that when the end-diastolic volume increases, the consequent stretch of the myocardial fibres generates a more forceful contraction. It has been shown that in the eel (*Anguilla anguilla*) heart, nitric oxide (NO) exerts a direct myocardial relaxant effect, increasing the sensitivity of the Starling response.

With the use of isolated working heart preparations, where cardiac loading and heart rate can be carefully controlled, the focus of the present study was to investigate the relationship between NO modulation of Starling response and temperature challenges in the eel. The results showed that while NO synthase inhibition by L-N5(1-iminoethyl)ornithine significantly reduced the Frank-Starling response when the experiments were performed at the same acclimation temperature (i.e. 20°C or 10ºC), it was without effect when the experiments were carried out under thermal shock conditions (i.e. from 20°C to 15 or 10ºC; or from 10°C to 15 or 20ºC), suggesting that in *A. anguilla* NO modulation of the Starling response is abolished by an acute thermal change. Western blotting analysis revealed a decrement of peNOS and pAkt expression in samples subjected to thermal shock compared to the control (heart working at the same acclimation temperature). Moreover, an increase in Hsp90 protein levels was observed under heat thermal stress.

Taken together, these data suggest that the NO synthase–NO-dependent modulation of the principal cardiac regulatory mechanism in fish is sensitive to thermal shock.

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Poster Session – Sunday 1st July 2012

A13.76

Impact of a single nucleotide polymorphism in PPARδ gene on training response and muscle fibre types, fine structure and physiology

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The peroxisome proliferator-activated receptor δ (PPARδ) transcription factor is an essential regulator of mitochondrial activity and oxidative capacity in skeletal muscle. Over-expression of PPARδ leads to increased fatty acid oxidation and improves insulin sensitivity. By contrast, a single nucleotide polymorphism (SNP) rs2267668 A/G in the PPARδ gene has been suggested to impair insulin sensitivity and aerobic physical fitness.

Following the hypothesis that persons affected by this SNP should have reduced exercise trainability, we investigated the responses of sedentary men and a control group to endurance training (cycling, 3 × 60 minutes/week, 10 weeks, HR@70–90% V_{O2peak}). Our results show that persons with the rs2267668 A/G SNP do indeed have a reduced response to endurance training at the sub-maximal performance level compared to the control group. However, quantitative assessment of capillary supply, mitochondrial density and of slow contracting oxidative fibre content in the vastus lateralis muscle indicates significant increases in these variables in both the SNP and the control groups.

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Poster Session – Sunday 1st July 2012

A13.77

AquaResp® — free open-source software for measuring oxygen consumption of resting aquatic animals

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AquaResp® is a free open-source software program developed to measure the oxygen consumption of aquatic animals using intermittent flow techniques. This free program is based on Microsoft Excel, and uses the MCC Universal Library and a data acquisition board to acquire analogue readings from up to four input ports and output control via two digital and two analogue ports. In addition AquaResp can read one COM-port if the oxygen analyser has a RS-232 output signal.

The present version of the program has options for parsing data strings generated by two major fibre optic oxygen electrode manufacturers. AquaResp was developed with the intention of automating data acquisition and control by programming in commonly-available software (Microsoft Excel) and allowing customization by the user without restrictions. The program has been tested in different laboratories for an extended period

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Poster Session – Sunday 1st July 2012

A13.78

In vivo near real time imaging of oxygen partial pressures in the glass catfish (*Kryptopterus bichirris*)

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By injecting 1 µm microspheres containing an oxygen-dependent phosphorescent dye into the vascular system and tissue of the transparent glass catfish (*Kryptopterus bichirris*), it is possible to measure near real-time oxygen partial pressure *in vivo*. We used a commercially-available digital single-lens reflex camera mounted with an optical long pass filter (λ = 490 nm) and excited the phosphorescent dye in the microspheres inside the fish with externally-mounted blue light emitting diodes (λp = 470 nm) to image the oxygen partial pressure. This method makes it possible to investigate oxygen partial pressures in the vascular system and different tissues of fish without having to insert any probes into the animal.

After injection of the microspheres and a recovery period from the anaesthesia, *in vivo* oxygen partial pressure can be determined by just taking a picture of the live fish exposed to blue light. As no electrodes or sensors are attached, the method allows a wide range of experiments investigating *in vivo* oxygen levels under different environmental perturbations.

This poster will show results and method.

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Poster Session – Sunday 1st July 2012

A13.79
Seasonal stage differences overwhelm environmental and individual factors as determinants of energy expenditure in free-ranging red squirrels

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Despite the central importance of the rate of energy expenditure in the lives of animals, the major drivers of within-species variation in energy expenditure remain uncertain, largely because most intra-specific studies focus on one, or only a few, potential determinants of expenditure. Here, we examine the determinants of daily energy expenditure (DEE) in free-ranging female North American red squirrels (*Tamiasciurus hudsonicus*) occupying a highly seasonal environment.

By relating variation in 260 measurements of DEE from 176 individuals to key sources of seasonal (reproductive and foraging seasonal stages), environmental (resources and air temperature) and individual (body mass and individual identity) variation, our comprehensive analysis examines the relative importance of DEE predictors that have been more commonly examined in isolation.

Seasonal stage differences accounted for most variation in DEE, with high expenditure during lactation and autumn hoarding, and very low expenditure during winter. Contrary to inter-specific studies, energy expenditure increased with increasing ambient temperature and it was weakly related to body mass in all seasons except for winter. High resource availability was associated with reduced energy expenditure in winter, but elevated expenditure during lactation and hoarding.

Collectively, these results highlight substantial intra-specific variation in energy expenditure, most of which can be explained by a combination of seasonal stages and environmental conditions, and fundamental differences in the importance and direction of determinants of energy expenditure when examined at the intra- *versus* the inter-specific level.

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Poster Session – Sunday 1st July 2012

A13.80
Z-disk related *post-mortem* degradation of skeletal muscle – a multi methodological approach

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In course of a study on degradation patterns of skeletal muscle *post-mortem*, the changes in muscle fibres were analysed. Muscle cells or fibres are composed of myofibrils, which are built up of a series of sarcomeres, representing the smallest functional units. Contractile protein forms actin and myosin filaments within sarcomeres and yet myofibrils are anchored primarily in a discoid-shaped dense band of proteins networked to form a small square lattice, thus referred to as Z-disk. A number of studies suggest a correlation of Z-disk structure integrity and meat quality (tenderness).

In the present study, rat skeletal muscle samples were dissected at predefined selected points of time *post-mortem*. Findings using electron microscopy techniques, such as formation of intermyofibrillar gaps, and increased width and the amorphous appearance of Z-disks, initiated further biochemical experiments via sodium dodecyl sulphate polyacrylamide gel electrophoresis and Western blotting. They also led to immunocytochemical analysis via fluorescence microscopy. Subsequently, individual methodological results have been analysed and discussed.

Among other proteins, desmin – as an important cytoskeletal element that laterally interconnects Z-disks and acts as a biomechanical scaffold for myofibrils – appears to play a major role in the postmortal Z-disk degradation processes.

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Poster Session – Sunday 1st July 2012

A13.82
Post-conditioning with catestatin in the isolated hearts of spontaneous hypertensive rats: Role of the RISK pathway

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The plasma levels of chromogranin A-derived peptide, catestatin, are decreased in hypertensive patients and in their offspring. Exogenous catestatin rescues the arterial hypertension of chromogranin A knockout mice. Ischemic post-conditioning, a therapeutic intervention in the early phases of reperfusion, limits reperfusion injury. Post-conditioning reduces infarct size via the reperfusion-injury-salvage-kinase (RISK) pathway.

In the presence of hypertension post-conditioning cardioprotective effects are blunted. We aimed to study whether catestatin post-conditioning is protective in a hypertensive model. The effects of catestatin post-conditioning on infarct size and cardiac function (developed left ventricular pressure) were studied in isolated hearts of both normotensive and spontaneously hypertensive mice (SHM) that underwent the following protocols:

- 30-minutes of ischemia and 120-minuts of reperfusion (I/R);
- post-conditioning (I/R plus five cycles 10-s I/R; and
- catestatin post-conditioning (I/R plus catestatin 75 nM for 20 minutes at the beginning of reperfusion).

Infarct size was 47 ±6% and 68±11% in normotensive I/R and SHM I/R, respectively. Post-conditioning significantly reduced infarct size in the normotensive (31 ±7%) mice, but not in SHM (58 ±7%). Catestatin post-conditioning, however, significantly reduced the infarct size in both strains. Post-ischemic recovery of developed left ventricular pressure was greater with catestatin post-conditioning than post-conditioning in both strains. While catestatin post-conditioning reduced contracture in SHM and normotensive mice, post-conditioning only reduced contracture in the normotensive nice. Co-infusion with specific inhibitors of the RISK pathway blocked the catestatin post-conditioning protective effects. Catestatin post-conditioning induced phosphorylation of RISK elements and affected HIF-1α distribution within cardiac tissues.

Here, we provide evidence for a hypertension-induced defect in the protective efficacy of post-conditioning that is overcome by catestatin.

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Poster Session – Sunday 1st July 2012

A13.83
Impaired chill tolerance in the digesting migratory locust (*Locusta migratoria*)

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A classical adaptation of freeze-avoiding insects is to void the intestinal lumen, eliminating ice nucleating activity. *Locusta migratoria* is a chill-susceptible insect (sensitive to low temperature), and its cold tolerance strategy is therefore not related to freeze avoidance. Nonetheless this study explored the supposed adaptive benefit of fasting with regard to chill tolerance, since the physiological challenge in digestion and cold might interact.

Earlier studies have demonstrated that high potassium loads in the diet increase haemolymph [K^+], causing depolarization of muscle cell resting potential, thus depressing muscle force development resulting in sluggish grasshoppers. Similarly it has been shown that low temperature increases haemolymph [K^+] due to a mismatch between active and passive transport processes between the haemolymph and intestinal tract.

The present study investigates cold tolerance and the regulation of ion homeostasis in fasted and fed grasshoppers in order to characterize the interplay between digestive K^+ loads, chill coma and K^+ imbalance. The hypothesis is that K^+-rich diets prior to a cold shock will exacerbate the subsequent K^+ imbalance, leading to increased chill injury and recovery time.

Preliminary results shows that the chill coma recovery time of grasshoppers fed on fresh wheat (high K^+) was 25% longer (14.78 ±0.55 m) compared to fasting grasshoppers (11.73 ±0.75 m) and grasshoppers fed on a no K^+ diet of sugar-paper (11.66 ±0.58 m) following a cold shock treatment at -4ºC lasting two hours (p=0.023). Current experiments aim to quantify the different treatment groups' ability to control and recover ion and water balance following cold shock.

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Poster Session – Sunday 1st July 2012

A13.84
Characterisation of a tonic muscle fibre population in the zebrafish axial musculature

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It has long been known that the skeletal muscle of teleost fish contains muscle fibres that are in all probability of a tonic type according to morphological criteria. The evidence for the existence of teleost tonic fibres, however, is still confined to a very small number of species, and knowledge concerning their ontogeny and possible functions is even more restricted. A remarkable deficit in this context is that it is not exactly known whether the zebrafish (*Danio rerio*), which is widely used to study vertebrate developmental biology, has such fibres or how they arise. In the present study we contribute to filling this gap by examining the existence of tonic fibres in the zebrafish myotome using a combined histochemical, immunocytochemical and ultrastructural approach.

Results demonstrate that a population of fibres, previously defined as 'red muscle rim'-fibres, can be attributed to the teleost tonic type. We show clearly that tonic fibre formation in the zebrafish myotome begins during midlarval life and is characterized by a preferred placement of such fibres within the dorsal part of the slow muscle domain, next to the horizontal septum. These findings are discussed in terms of their fit with the criteria used for tonic fibre identification in other species, their possible modes of formation, and in relation to a probable role of body length as a key determinant to induce this formation in the context of larval hydrodynamics.

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Poster Session – Sunday 1st July 2012

A13.85
Ketamine acute toxicity assessment using the zebrafish (*Danio rerio*) embryo toxicity test

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Recently, illicit drugs have been identified as wide-spread class of emergent contaminants in municipal wastewaters, which are of major concern to aquatic life. Ketamine, a dissociative anaesthetic, is currently used in human and veterinary medicines and has gained popularity as a drug of abuse. It has been detected in hospital effluents and river waters, reaching maximum concentrations of 0.4 mg/L. To evaluate the impact of acute ketamine exposure on fish, we used the zebrafish embryo toxicity test.

Zebrafish (*Danio rerio*) embryos (n=100/group) about two to three hours post-fertilization were exposed for 20 minutes to nominal concentrations of ketamine (0.2, 0.4 and 0.8 mg/L) or ethanol in a concentration of 2% (v/v) on four independent replicates. Toxicity (lethality, teratology and hatching) was assessed daily until 144 hours post-fertilization. Total malformation index (spine, fins, cranial/facial, thorax, abdominal and position in water column) was scored as degree (i.e. zero through four). Statistical analyses were performed using SPSS software.

At early stages (24–48 hours post-fertilization) no significant effects were observed. At the later stages (72–144 hours post-fertilization), the most noticeable effects were decreased hatching rate and the increased frequency and score of skeletal deformities (p<0.05). Exposure to ketamine increased the total index of malformation in a concentration-dependent manner (r=0.73, p=0.001).

The results show that exposure to ketamine during zebrafish embryogenesis has a potentially teratogenic effect. Furthermore, they highlight the continuing importance of studying the effects of emergent pollutants to understand how these might effect wildlife and ecosystems.

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Poster Session – Sunday 1st July 2012

A13.87
NOS-dependent 'on/off' switch and apoptosis in the freshwater and aestivating lungfish *P. annectens*: Skeletal *versus* cardiac muscle

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African lungfishes (*Protopterus* spp.) are obligate air breathers that aestivate during the dry season, undergoing prolonged skeletal muscle immobilization and cardiac readjustments. The molecular mechanisms underpinning aestivation and post-arousal recovery responses of cardiac and skeletal muscle are unknown. Using biochemical and immunofluorescence methods, we analysed the expression, localization and activity of the endothelial nitric oxide synthase isoform, eNOS, and its partners, Akt and Hsp90, in the cardiac and skeletal muscles of *Protopterus annectens* under three experimental conditions: freshwater, six months of aestivation (6mAe) and six days after arousal (6mAe6d).

The modulation of a functional eNOS/nitric oxide system in these two muscle tissues in response to environmental stress was tissue-specific. During aestivation, p-eNOS/eNOS and p-Akt/Akt ratios increased in the cardiac muscle and decreased in the inactive skeletal muscle,

although Hsp90 increased in both tissues. Analysis of cell turnover (TUNEL) showed that the apoptotic nuclei in the cardiac muscle remained comparable between the three conditions, but there was an increase in TUNEL-positive nuclei in the skeletal muscle during aestivation. Expression of apoptosis repressor with a caspase recruitment domain was strongly reduced in the skeletal muscle of 6mAe fish, but remained unchanged in the cardiac muscle of 6mAe and 6mAe6d fish in comparison with the freshwater, indicating a preserved cardiac activity during aestivation.

In conclusion, our results indicate the importance of the eNOS/NO system and cell turnover regulation in the morphofunctional readjustments of the cardiac and skeletal muscles of *P. annectens* during the switch from freshwater conditions to aestivation and from aestivation to arousal.

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Poster Session – Sunday 1st July 2012

A13.89

Cardiac structure and function in the goldfish (*Carassius auratus* L.) heart

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Using morphological and physiological approaches, for the first time we have provided a structural and functional characterization of *Carassius auratus* L. heart. Besides the classical four chambers, i.e. the sinus venosus, atrium, ventricle and bulbus, we have described two distinct structures corresponding to the atrioventricular 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 inner spongiosa; the bulbus wall is characterized by a high elastin-to-collagen ratio, which makes it extremely compliant. Immunolocalization revealed a strong expression of activated 'eNOS-like' isoforms both at the vascular endothelium and, to a lesser extent, in the myocardiocytes and the endocardial endothelium. 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 (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, with values higher than 1.5 kPa impairing its performance. These morphofunctional features are consistent with a volume pump mechanical performance.

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Poster Session – Sunday 1st July 2012

A13.90

Recovery of ion homeostasis following cold shock precedes chill coma recovery and resumption of mating behaviour in *Locusta migratoria*

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The chill tolerance of insects is a vital temperature response that markedly influences their ability to persist in cold environments. Mounting evidence indicates that chill tolerance is associated with the ability to maintain ion- and water-homeostasis, thereby ensuring muscular function and preventing chill injury. In the present study, the chill-susceptible locust, *Locusta migratoria*, was used to examine the relationship between the recovery of muscle and haemolymph ion homeostasis with the time to regain posture and reproductive behaviour following cold shock (two hours at -4°C). In addition, the effects of rapid cold-hardening (RCH) were examined by exposing half of the animals to 0ºC for two hours before being transferred to the cold shock treatment.

Cold shock elicited a doubling of haemolymph [K^+] and this disturbance was even greater in grasshoppers pre-exposed to RCH. Recovery of ion homeostasis was markedly faster in RCH-treated animals, however, to the extent that they had almost entirely re-established [K^+] homeostasis after 30 minutes. Recovery of ion homeostasis correlated well with whole-organism performance, as RCH-treated individuals regained posture faster than non-hardened individuals (13.7 ±0.6 minutes *versus* 22.7 ±1.4 minutes). Similarly, hardened individuals regained reproductive behaviour faster after cold shock, with an average time before mating attempts for hardened individuals being 33.8 ±3.4 minutes *versus* 73.7 ±10.0 minutes in non-hardened individuals.

These results demonstrate how chill coma recovery is directly related to the insect's ability to recover extracellular ion-balance. Moreover, we present correlative data to suggest that the benefits of RCH are achieved through an increased capacity for recovery of ion homeostasis.

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Poster Session – Sunday 1st July 2012

A13.91

Cloning and characterization of a novel family of phospholipase Cs in fish

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Phosphatidylinositol-specific phospholipase C (PI-PLC) enzymes comprise a small family of receptor-regulated phosphodiesterases that control many cellular processes by the regulation of cytosolic calcium and/or the activity of several protein kinases. Comparative microarray analysis between sexually immature 'yellow' eels and sexually mature 'silver' eels revealed differential expression of a novel PI-PLC family member known as phospholipase C X-domain-containing protein (PLCXD). Homologues of this novel gene have been identified in the genomes of all species investigated thus far, from bacteria to man.

Although only one isoform has been identified so far in the eel (*ePLCXD*), at least five isoforms are present in zebrafish (*zfPLCXD1—5*). Real-time polymerase chain reaction and quantitative polymerase chain reaction analyses revealed zebrafish *PLCXD* genes to be distinctive with respect to their tissue distribution patterns, suggesting that all five zebrafish isoforms possess unique physiological functions. In particular, *zfPLCXD3* was found to be highly expressed in the heart and subsequent morpholino knockout of *zfPLCXD3* in zebrafish embryos resulted in dilatation and abnormal folding of the heart, which was accompanied with slower heart rates and pericardial oedema. The *ePLCXD* isoform was found to be highly expressed in the intestine of the yellow eel compared to silver eels, suggesting a potential developmental role. A phylogenetic analysis revealed the different *zfPLCXD* isoforms to be highly conserved across species, which is highly suggestive of essential functions. Interestingly, the *ePLCXD* isoform did not group with *PLCXD* orthologues in other teleost fish.

The functional significance of these results remains to be determined.

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Poster Session – Sunday 1st July 2012

A13.92

Partition of aerobic and anaerobic swimming costs and their correlation to tail-beat frequency and burst activity in *Sparus aurata*

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Aerobic and anaerobic oxygen consumption was measured for 13 *Sparus aurata* swimming at incremental increasing swimming speeds until fatigue at 10ºC. The anaerobic swimming cost was measured as the excess post-exercise oxygen consumption (EPOC) following each swimming speed. To determine tail-beat frequency, amplitude and burst and coast behaviour, the peduncle position was determined at 25 s^{-1} by video tracking.

The data showed that *S. aurata* swam exclusively using aerobic metabolism up to 88% of their critical swimming speed (U_{crit}). Above 88% U_{crit}, the contribution from the anaerobic swimming cost increased exponentially and represented 12%, 59% and 70% of the total swimming cost at 90%, U_{crit} and fatigue, respectively, and resulted in a total anaerobic capacity of 170 mg O_2 kg^{-1}. Normalized tail-beat amplitude and frequency both predicted the swimming speed but only tail-beat frequency was able to predict the aerobic swimming cost. The change to burst and coast swimming was correlated to the first measurements of EPOC and both the burst frequency (bursts min^{-1}) and burst distance (percentage burst distance) were found to predict EPOC by linear regressions. The low temperature used in the present study resulted in a prolonged recovery time, which increased with the anaerobic contribution to 10 hours after fatigue. Due to the late initiation time, the contribution from the anaerobic swimming cost did not affect the optimal swimming speed or the minimum cost of transport. In contrast, the cost of swimming above 88% U_{crit} increased by 334% at fatigue.

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Poster Session – Sunday 1st July 2012

A13.93

Copper induced up-regulation of apoptosis genes in zebrafish gill

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Zebrafish (*Danio rerio*) has long been used as a model in toxicological studies to study the effects of different classes of environmental contaminants, such as heavy metals. In the present work, adult zebrafish were acutely exposed to waterborne copper in order to evaluate the apoptosis induction in gill, an important organ of copper uptake and the first target of its toxicity. Four genes were selected to identify copper-activated apoptosis pathways: *caspase-8* (extrinsic pathway), *caspase-9* (intrinsic pathway), *apoptosis inducing factor* (*AIF*; caspase-independent) and *p53*. Zebrafish were exposed to 12.5 and 100 µg/L of copper, for six, 12, 24 and 48 hours. Fish gills (n=6) were collected to undergo gene expression analysis through real-time polymerase chain reaction. Relative gene expression was determined by the $\Delta\Delta C_T$ method and the values were normalized to the control average value.

Copper exposure induced the up-regulation of the four genes studied in zebrafish gills. Their up-regulation was independent of the copper concentration and occurred at different time points during the exposure period. The first gene to be up-regulated was *p53*, at six hours, followed by *caspase-9* at 12 hours, *caspase 8* at 24 hours and *AIF* at 48 hours.

The results suggest that the copper induction of apoptosis in zebrafish gill is initiated via an intrinsic pathway, through p53 activation and is followed at 24 hours by the extrinsic pathway. The caspase-independent pathway is the last to be activated, as suggested by the up-regulation of AIF at 48 hours of exposure.

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Poster Session – Sunday 1st July 2012

A13.94

Gas exchange in rodent burrows depends on transient, turbulent air flow and eddies at the ground surface

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Burrows are the living environment for numerous animal species. The conventional approach to burrow ventilation involves two main mechanisms: diffusion of gases through the soil, and forced convection driven by pressure differences between openings. Visual observation of smoke patterns did not conform to what would be expected if diffusion of gasses was the dominant mechanism. This led us to hypothesize that ventilation could be the result of the occasional penetration of eddies.

To test this hypothesis, we positioned thermocouples along a burrow made of PVC. Changes in air temperature were observed to occur sequentially along the burrow, starting at the entrance. These changes were sporadic and unpredictable, suggesting that the hypothesis put forward is correct.

A direct result of this is that diffusion did not play a major part in ventilation where the burrow was affected by eddy penetration. It can, however, play a major role in dead-end spaces. To test this mechanism, we injected CO_2 into the nest chambers of mesh-cloth burrows, and then measured CO_2 concentrations in the upper part of the nest chamber. We compared CO_2 concentrations between burrows with and without free exchange of gas through the soil matrix while their entrances were either open or plugged. We found that diffusion through the soil is not negligible in dead-end spaces and is negatively correlated with wind speed.

Our results indicated that even when above-ground wind speed was low, CO_2 concentrations in the nest chamber did not reach physiologically deleterious levels.

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Poster Session – Sunday 1st July 2012

A13.95

Ultrastructural adaptations to endurance exercise in domestic dogs

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Endurance exercise training enhances the oxidative capacity of muscles in dogs. We measured the effects of seven weeks of treadmill training and their reversibility after stopping training in a group of foxhound-boxer-Ingelheim Labrador mixes. We analysed light microscopy images and transmission electron microscope images of microbiopsies of the M. adductor magnus of dogs three times: before the training started, in trained condition, and nine weeks after the end of training. Using standard morphometrics, we measured decreased myofibre diameter in the muscles of dogs in trained condition (0.040 ±0.007 mm) when compared to the untrained condition (0.049 ±0.004 mm).

No differences in myofibre diameter were found in the muscles of dogs in trained condition or nine weeks after training stopped (0.041 ±0.006 mm). Applying stereology, we found that training led to increases

in the area occupied by mitochondria within myofibres. Training also led to an increase in the proportion of christae within the mitochondria. As an index of changes of the mitochondrial network, we obtained the ratio of the circumference to the area of the mitochondria. In response to training, the ratio increased from 0.94 ±0.03 to 1.07 ±0.09. Besides the decrease in myofibre diameter, all of the reported changes were reversible and measurements nine weeks after the training stopped did not differ from measurements before training.

The results of this study emphasize the ability of the locomotor muscles of dogs to rapidly and flexibly adapt to changing exercise regimes by up- and down-regulation of the amount and conformation of their mitochondria.

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Poster Session – Sunday 1st July 2012

A13.96
Cardio-respiratory effects of autologous blood doping in the Antarctic fish *Pagothenia borchgrevinki*

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The Antarctic red-blooded *Pagothenia borchgrevinki* have an extreme capacity for autologous blood doping and have the capability to increase their haematocrit to approximately two to four times higher than other species studied. The general concept is that due to the low temperature and the presence of antifreeze proteins, the viscosity of the blood is high. The largest contribution to blood viscosity is the addition of erythrocytes. Keeping the haematocrit low during periods with a low oxygen demand would minimize the time with high viscosity.

The present study concentrated on the cardiorespiratory effects of this large increase in haematocrit during exercise and how this affects the cardiorespiratory variables: oxygen consumption, ventral aortic blood pressure, cardiac output, heart rate and stroke volume.

The significance of the increase in haematocrit on the oxygen-carrying capacity of the blood was apparent in placebo-operated fish, as their oxygen consumption during exercise was significantly higher compared to a group with ligated spleens. The increase in haematocrit was also associated with increased working load of the heart, as the systemic blood pressure was significantly increased in the placebo-operated group compared with the group with ligated spleens. Despite the differences in haematocrit and blood pressure, no differences between the two groups were found in cardiac output, heart rate or stroke volume. The results demonstrate both the cost and benefit of a high haematocrit, and thus the capacity to store a blood reserve in the spleen is beneficial for this cold-living species.

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Poster Session – Sunday 1st July 2012

A13.97
GLUT transporter expression and capacities for fuel uptake in the muscles of hovering hummingbirds

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Most mammals fuel low-intensity exercise largely with fat and rely increasingly on carbohydrates for fuel as exercise intensity climbs. This conserved phenomenon, termed the 'crossover concept', does not apply to hummingbirds and some species of nectarivorous bats.

Hummingbirds fuel energetically-demanding hovering flight almost entirely with fat when fasted and exclusively with carbohydrates when fed. The switch from fat to ingested sugars as fuel occurs rapidly and is, in part, enabled by adaptations in digestive physiology that permit rapid sugar absorption.

The mechanisms that control fuel selection at the level of muscle tissue are not well understood. Hummingbirds experience large swings in blood sugar levels as they transition from a fasted to fed state, but blood sugar levels in fasted birds are nevertheless higher than in comparably sized mammals. In mammals, increasing contractile activity results in the movement of GLUT4, the insulin-responsive glucose transporter, from intracellular vesicles to the muscle cell membrane. This shift in sugar uptake capacity by muscle fibres with increasing exercise intensity is thought to partially underlie the observed changes in fuel use. We hypothesized that hummingbirds lack a comparable 'exercise-sensitive' GLUT protein and that this partly explains why the 'crossover concept' does not apply to them. Analysis of GLUT family mRNA and protein expression confirms the absence of a GLUT4 homologue.

These findings raise intriguing questions regarding the convergence of patterns of fuel use in hummingbirds and nectar bats, despite probable differences in the regulation of sugar transport capacity at the level of muscle tissue.

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Poster Session – Sunday 1st July 2012

A13.98
The effects of temperature on E-C coupling and intracellular Ca^{2+} buffering in trout cardiomyocytes

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We used whole-cell voltage clamping and Fura-2 photometric techniques to investigate the effects of acute temperature change on excitation–contraction coupling and cytosolic calcium (Ca^{2+}) buffering in isolated atrial and ventricular myocytes at 7°C, 14°C and 21°C from rainbow trout acclimated to 12°C.

In both atrial and ventricular myocytes, temperature did not affect Ca^{2+} transient ($[Ca^{2+}]_i$). Peak Ca^{2+} current density (I_{Ca}) decreased with decreased temperature. Gains in I_{Ca} inactivation time and sarcoplasmic reticulum Ca^{2+} load were highest at 7°C.

In atrial myocytes at 7°C and ventricular myocytes at 21°C, sarcoplasmic reticulum inhibition with thapsigargin (2 eM) and ryanodine (10 μM) decreased $[Ca^{2+}]_i$ and increased the slope when voltage-clamped at 0 mV for 500 ms. Inhibition of the sarcoplasmic reticulum decreased gain at 7°C and 21°C in atrial myocytes and at 21°C in ventricular myocytes. When voltage-clamped at 0 mV for 250 ms, a more physiologically relevant pulse duration, sarcoplasmic reticulum inhibition decreased $[Ca^{2+}]_i$ amplitude and gain in both cell types. Atrial myocytes also showed reduced rise in $[Ca^{2+}]_i$ slope.

The results show that $[Ca^{2+}]_i$ is kept independent of temperature, despite temperature-dependent decreases in I_{Ca}, by increasing gain, which might be caused by increased sarcoplasmic reticulum Ca^{2+} load. The relatively low, but temperature-independent K_d of the cytosol for Ca^{2+} (40–470 uM) is consistent with the relatively long relaxation times and low maximal heart rates of teleosts.

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Poster Session – Sunday 1st July 2012

A13.99

Neuromuscular control of aerodynamic power output via changes in wing-beat kinematics in the flight muscles of ruby-throated hummingbirds (*Archilochus colubris*)

Sajeni Mahalingam (University of Toronto, Ontario, Canada) and Kenneth Welch (University of Toronto, Ontario, Canada)

Hummingbirds have one of the highest wing-beat frequencies of any flying vertebrate, yet they are able to modulate aerodynamic power output precisely. By examining how electromyograms and the wing-beat kinematics of hummingbirds change as aerodynamic power requirements for hovering flight are altered by the addition of weights or by varying the density of the air, we can better understand how aerodynamic power output is modulated via neuromuscular control.

Birds generate lift predominantly during the down-stroke (powered by the pectoralis). However, hummingbirds are unusual in their ability to generate three to four times more lift during the upstroke (powered by the supracoracoideus) compared to other birds. Despite this relative symmetry of wing-beat half strokes, previous work indicated that the timing of supracoracoideus activation in relation to upstroke varied with forward flight speed.

Variation in the timing and intensity of activation of the supracoracoideus has not been thoroughly examined during hovering. We predicted that, unlike during forward flight, the pectoralis and supracoracoideus would show similar patterns of activation as lift requirements varied during hovering.

Results from load lifting trials and hypodense trials in ruby-throated hummingbirds (*Archilochus colubris*) indicate that they increase aerodynamic power output by progressively increasing both wing-beat frequency and stroke amplitude. The increase in wing-beat frequency and stroke amplitude correlates with increases in the number of spikes per wing-beat, spike amplitude, and rectified electromyogram area to similar magnitudes in the pectoralis and supracoracoideus muscles. This suggests that the relative symmetry in lift production between half wing-beats is driven by matched variation in recruitment patterns.

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Poster Session – Sunday 1st July 2012

A13.100

Evidence of selection pressure within multiple toll-like receptor 22 paralogues in Atlantic cod

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Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns and elicit specific immune responses to protect the host from potential pathogen invasions. To date, 23 TLRs have been reported in various organisms including plants, mammals, birds and fish. Many TLRs have been commonly retained across all species, while others were lost, gained or diverged independently throughout evolution. Teleost fishes possess distinct TLRs – *tlr21*, *tlr22* and *tlr23* – for which no functional orthologues have been identified in mammals.

The recently-published genome sequence of Atlantic cod (*Gadus morhua*) revealed a remarkable expansion of MHC I genes and TLR families. The genome encoded for high number of teleost *tlr22* genes, while most mammalian homologues were absent. The cod *tlr22* paralogues coded for putative proteins that shared up to 78% sequence identity with each other and up to 54% with other known teleost Tlr22 proteins.

Phylogenetic analysis of all teleost *tlr22* genes grouped the cod *tlr22* paralogues under a single clade, indicating that they might have arisen through lineage-specific duplications. Selection pressure analysis using phylogenetic analysis by maximum likelihoo identified 42 codons to be under positive selection, detected by both M2 and M8 models in naïve empirical Bayes analysis with a dN/dS ratio of 3.34 and 3.23, respectively. Most of the codons that were found to be under positive selection code for amino acids that are involved in pathogen recognition.

It is likely that positive natural selection is shaping the evolutionary pattern of fish-specific TLRs, which might be associated with adaptation to new habitats and rapidly-evolving pathogens.

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Poster Session – Sunday 1st July 2012

A13.101

In vitro O_2 affinity in normoxia, hypoxia and hypercapnia during development in American alligator, *Alligator mississippiensis*

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Developing American alligator (*Alligator mississippiensis*) embryos can experience hypoxia and hypercapnia during incubation in natural mound nests. This can compromise haemoglobin saturation during periods of acute decreased nest $P_{O2,}$ as occurs following increased nest matrix water content after rainfall. Alligator egg porosity and gas permeability also increase throughout incubation, maximizing gas flux prior to hatching.

We measured the *in vitro* oxygen binding affinity of embryonic alligators at 70% and 90% of development incubated in normoxia, hypoxia or hypercapnia at 30 and 35°C. Embryonic P_{50}s declined between these points in development, similar to developmental changes documented in saltwater crocodiles. Chronic hypoxic incubation did not significantly shift embryonic P_{50}s, but chronic hypercapnic incubation lowered 70% and 90% P_{50}s. Chronic hypoxic incubation had no effect at either developmental stage.

Our findings suggest that haemoglobin can change in embryonic alligators, as it does in foetal mammals, which results in different O_2-binding properties relative to the adult phenotype.

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Poster Session – Sunday 1st July 2012

A13.102

How ubiquitous is endothelial nitric oxide synthase?

Stuart Egginton (University of Birmingham, UK), Fahima Syeda (University of Birmingham, UK), David Hauton (University of Birmingham, UK) and Steven Young (University of Birmingham, UK)

The role of endothelial nitric oxide synthase (eNOS)-derived nitric oxide (NO) for vasodilatation and/or angiogenesis in vertebrates is equivocal. A lack of eNOS-specific inhibitor suitable for *in vivo* use means that it is difficult to ascertain the importance of this NOS isoform in animals apart from mice, where gene ablation has shown that eNOS, but not neuronal (nNOS), is essential in the regulation of vascular tone, control of blood pressure and angiogenesis. Whether this phenomenon exists in other vertebrates is unclear. There is no direct evidence that eNOS exists in some fish species including rainbow trout, common carp and zebrafish.

Although non-specific NOS blockade with L-NNA inhibits angiogenesis in rainbow trout (unpublished data) and reduces blood flow in zebrafish, whether the eNOS isoform is responsible has not been established. Further, isolated carp vessels do not dilate in the presence of sodium

nitroprusside or exogenous cyclic guanidine monophosphate (cGMP), suggesting that the vascular NO-cGMP pathway does not exist in this species. There are no sequences in the literature for an eNOS gene in any fish species, and polymerase chain reaction performed on three fish species produces differing products.

Blood pressure in fishes is lower than in mammals, suggesting that potent vasodilator activity exists. Hence, the role of eNOS in blood flow and angiogenesis might not be the same in all vertebrates. It is unclear when the NOS3 gene appeared during vertebrate evolution, why/if NOS3 activity is advantageous, and which alternatives permit sensitive regulation of microvascular function in non-mammalian vertebrates.

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Poster Session – Sunday 1st July 2012

A13.103

Aerobic scope as an explanation of variation in inter-individual growth in the spotted wolffish (*Anarhichas minor*)

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Variability in growth rate is a common finding in fish populations. Recent work has looked for physiological differences that might explain the differences in traits such as growth rate. Due to the dependence of growth on energy acquisition, we investigated possible links between standard metabolic rate (SMR), maximum metabolic rate (MMR), the aerobic scope (AS = MMR – SMR) and the growth rate of spotted wolffish.

A previous growth experiment (125 days, 8°C, *ad lib* food availability) resulted in the selection of the 20 best and worst growing fish (SGR = 1.23 ±0.048 and 0.945 ±0.066 g, mean ± standard deviation [SD]. These very different fish did not, however, differ in SMR (overall mean ± SD: 31.46 ±4.99 $mg{\cdot}h^{-1}{\cdot}kg^{-1}$), MMR (102.33 ±16.07 $mg{\cdot}h^{-1}{\cdot}kg^{-1}$) nor AS (70.88 ±13.89 $mg{\cdot}h^{-1}{\cdot}kg^{-1}$).

Hypoxia limits MMR in fish, so the physiological traits resulting in fast growth could be different in normoxia. The growth experiment also had a 40% air saturation treatment from which 20 fast and slow growers were selected (SGR = 0.77 ±0.053 and 0.50 ±0.073 g). The same physiological variables were tested in normoxia. The critical O_2 value (O_{2crit}) was also measured. Again, we could not find any difference in SMR (36.32 ±5.045 $mg{\cdot}h^{-1}{\cdot}kg^{-1}$), MMR (155.81 ±19.54 $mg{\cdot}h^{-1}{\cdot}kg^{-1}$), AS (79.49 ±17.09 $mg{\cdot}h^{-1}{\cdot}kg^{-1}$) or even O_{2crit} (35.38 ±5.90%sat) between these two groups of fish.

Other variables, possibly SDA, are responsible for differential growth rate in this species.

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Poster Session – Sunday 1st July 2012

A13.104

Role of orexinergic neurotransmission in the respiratory response to hypercarbia and hypoxia in toads

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The hypocretin or orexin A (OX) plays an important role in the modulation of respiratory control in mammals, but there are no data available for the role of OX-LC in the peripheral and central chemoreception of amphibians. Thus, the present study was designed to investigate whether the orexinergic system of unanaesthetized toads (*Rhinella schneideri*) is important to hypoxic (5% O_2 and N_2 for balance) and hypercarbic (5% CO_2, 21% O_2 and N_2 for balance) chemoreflex.

We assessed the role of the orexinergic system on respiratory responses by using intracerebroventricular injection of SB-334867 (the orexin A receptor antagonist OX-AR). To this end, a guide cannula was implanted into the lateral ventricle of the brain so that injections of the antagonist or vehicle could be given. Animals were maintained at 25°C during all of the experiments.

Our results demonstrated that injection of OX-AR antagonist attenuated the increase of the ventilatory response to hypercarbia, by acting on tidal volume. The OX-AR antagonist did not, however, affect ventilation under hypoxic conditions. We conclude that central OX-AR contributes to hypercarbic but not to hypoxic chemoreflex in toads.

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Poster Session – Sunday 1st July 2012