



## Society for Experimental Biology Annual Main Meeting 28th June – 1st July 2009, Glasgow, UK

### A5 – GENERAL ANIMAL BIOLOGY

#### A5.1

09:00 Sunday 28th June 2009

**Follow your nose: Chemical communication throughout the European eel (*Anguilla anguilla*, L.) life-cycle**

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European eels are important in both scientific and economic terms. Unfortunately, current populations are becoming increasingly endangered and urgent management is needed. Several aspects of eel biology, together with their highly developed sense of smell, suggest that chemical communication could be involved at key stages of their life-history. Thus, advances in this area could generate novel tools for stock management.

Eels have acute olfactory sensitivity to substances released by conspecifics. Both the bile fluid and skin mucus have been implicated as routes of release for these odorants. Furthermore, the nature of these odorants depends on both the sex and reproductive status of the donor. Exposure to water conditioned by mature con-specifics causes stimulation of sexual maturation in immature eels. This is consistent with a role for chemical communication during reproduction. Also, eels can detect bile acids from other species; this may be involved in prey search and/or predator avoidance. Bile acids may well be important odorants in this respect; eels have higher sensitivity for sulphated C27 'ancient' bile acids rather than C24 'modern' forms. Our last study demonstrated different olfactory sensitivity to several odorants according to the environment and reproductive status. This may reflect changes in diets between seawater and freshwater eels and/or the changing role of chemical communication during different life-stages (e.g. maturation and migration). We propose the eel as a model species for studies on olfaction and chemical communication in fish because of its ancestral status in teleost evolution, its high olfactory sensitivity and its physiological plasticity.

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#### A5.2

09:20 Sunday 28th June 2009

**Functional asymmetry in the olfactory system of a flatfish, the Senegalese sole (*Solea senegalensis*)**

Zélia Velez (Centro de Ciências do Mar), Peter C. Hubbard (Centro de Ciências do Mar), Kevin J. Welham (University of Hull), Eduardo N. Barata (Centro de Ciências do Mar), Jörg D. Hardege (University of Hull), Adelino V. Canário (Centro de Ciências do Mar)

The two olfactory epithelia of the family Soleidae are exposed to different water sources; the upper (right) epithelium samples the water column whereas the lower (left) samples interstitial water. Given that one of the major prey species of the Senegalese sole (*Solea senegalensis*) is the ragworm *Hediste diversicolor* which lives in the substrate, we hypothesized that the lower epithelium may have evolved sensitivity to odorants released by the ragworm whereas the upper epithelium may be more involved in chemical communication and therefore is more sensitive to odorants released by conspecifics. Using a combination of solid-phase extraction, liquid chromatography linked to mass spectroscopy and electrophysiological recording from the olfactory epithelia (the electro-olfactogram), we identified a prey-related odorant (the aromatic amino acid, l-phenylalanine) and a conspecific-related odorant (the bile acid, taurocholic acid). The lower epithelium was significantly more sensitive to the former whereas the upper was more sensitive to the latter. Furthermore, cross-adaptation and pharmacological blockade of adenylate cyclase (SQ-22536) and phospholipase C signaling pathways (U73122) suggested that the receptors and transduction mechanisms involved, respectively, are different in the two epithelia; specific receptors for l-phenylalanine in the lower epithelium act *via* the phospholipase C pathway whereas specific receptors for taurocholic acid in the upper epithelium act *via* both adenylate cyclase and phospholipase C mediated pathways. These results are consistent with our hypothesis and suggest a functional asymmetry in the olfactory system of this flatfish.

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**A5.3****09:40 Sunday 28th June 2009****Analysis of domain functions in duplicated teleost glucocorticoid receptors (GRs) reveals the evolution of a glucocorticoid resistant GR in salmonids**

Armin Sturm (Institute of Aquaculture, University of Stirling), Nicolas R. Bury (King's College London)

Glucocorticoids are involved in the regulation of many physiological functions, including energy metabolism, immune function, growth and development. In teleost fish, the 'glucocorticoid' cortisol has further important roles in osmoregulation. Most of the effects of cortisol are mediated through the glucocorticoid receptor (GR), a ligand-dependent transcription factor regulating the expression of cortisol target genes. Many teleosts possess two GRs, which have a common evolutionary origin. Retention of duplicated GRs in many teleosts suggests an evolutionary benefit, implying neo- or sub-functionalisation. Indeed, the two GRs of rainbow trout, called rtGR1 and rtGR2, differ markedly (up to 100-fold) in hormone sensitivity. Here, we provide a detailed analysis of domain function in rtGR1 and rtGR2, based on different mutational strategies, including the generation of domain-swaps between rtGR1 and rtGR2, as well as N- and C-terminal truncations and single amino acid mutants. Hormone sensitivity was ruled by both the N-terminal A/B-domain, in which we mapped a major transactivation function, and the C-terminal E-domain, known to bind the hormone. Surprisingly, transactivation functions tau2 and AF2 of the E-domain of GR, as well as amino acids involved in hormone binding, could be ruled out as major determinants of the difference in sensitivity between rtGR1 and rtGR2. Instead, a unique C-terminal extension of the GR amino acid sequence, present only in rtGR1 and certain other salmonid GRs, was found to effect rtGR1's low hormone sensitivity. However, this effect was highly context dependent, requiring other parts of the rtGR1 E domain.

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**A5.4****10:00 Sunday 28th June 2009****Is the 5-HT<sub>2A</sub> receptor involved in mediating pulsatile urea excretion in gulf toadfish, *Opsanus beta*?**

M. Danielle McDonald (RSMAS, University of Miami), Lea R. Medeiros (RSMAS, University of Miami), Edward M. Mager (RSMAS, University of Miami)

The pulsatile urea excretion mechanism of the gulf toadfish is regulated by the stress hormone, cortisol, and by the neurotransmitter, serotonin (5-HT; 5-hydroxytryptamine). Specifically, high circulating concentrations of cortisol appear to inhibit pulsatile urea excretion while high levels of 5-HT are stimulatory. Arterial injections of the mammalian 5-HT<sub>2</sub> agonist,  $\alpha$ -methyl 5-HT, results in urea pulses of natural size within 5 min of injection, suggesting the involvement of 5-HT<sub>2</sub> receptors in toadfish urea transporter (tUT) activation. Urea pulses elicited by  $\alpha$ -methyl 5-HT are inhibited in a dose dependent fashion by the 5-HT<sub>2</sub> antagonist ketanserin ( $IC_{50}$  = 0.0095  $\mu$ M) and based on the differential binding affinities of the three 5-HT<sub>2</sub> subtypes for  $\alpha$ -methyl 5-HT and ketanserin established for mammals, our results suggest the involvement of 5-HT<sub>2A</sub> in the mediation of pulsatile urea excretion by gulf toadfish. We have now cloned and sequenced the full-length 5-HT<sub>2A</sub> receptor from

toadfish brain, which shows >70% sequence homology to mammalian 5-HT<sub>2A</sub> receptors on the amino acid level. Injection of toadfish 5-HT<sub>2A</sub> cRNA into *Xenopus* oocytes results in a 3.5-fold increase in the binding of [<sup>3</sup>H]-5-HT compared to water-injected controls. Current work is investigating the specificity of the mammalian pharmacological compounds,  $\alpha$ -methyl 5-HT and ketanserin, for the toadfish 5-HT<sub>2A</sub> receptor using the *Xenopus* oocyte approach. Furthermore, we are using quantitative PCR to determine the tissue distribution of the receptor and investigate whether, like tUT, mRNA expression of the toadfish 5-HT<sub>2A</sub> receptor is sensitive to changes in circulating levels of cortisol.

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**A5.5****10:45 Sunday 28th June 2009****Characterizing and localizing the 5-hydroxytryptamine (5-HT, serotonin) subtype 1A (5-HT<sub>1A</sub>) receptor in *Opsanus beta*: Determining its role in the etiology of stress and depression**

Lea R. Medeiros (RSMAS, University of Miami), Ed.M. Mager (RSMAS, University of Miami), M.D. McDonald (RSMAS, University of Miami)

Injection with 8-hydroxy-2-(di-n-propylamino) tetraline (8-OH-DPAT), a 5-HT<sub>1A</sub> agonist in humans, causes a significant increase in circulating levels of cortisol within 0.25 h in the gulf toadfish, *Opsanus beta*. This suggests that the toadfish receptor, like the receptor in mammals, regulates the endocrine axis that controls the release of stress hormone. Whole-brain homogenate 5-HT binding assays were performed to determine protein binding function, and 5-HT receptors were found to be saturable at 5-HT concentrations <2.5 nmol l<sup>-1</sup> with a  $K_D$  of  $1.35 \pm 0.17$  nmol l<sup>-1</sup> ( $r = 0.996$ ,  $P < 0.001$ ); similar to findings in other fish and mammalian brains. The full length toadfish 5-HT<sub>1A</sub> cDNA was cloned and sequenced (Accession # FJ769221), showing 67.7 homology to the mammalian receptor. Using quantitative PCR, the receptor was found to be expressed in the intestinal tract, in low amounts in the liver, gill, heart, and muscle and predominantly in the brain, swim bladder, and gonad, but is not expressed in the kidney. To localize the receptor on a finer scale, the brain was dissected into five regions and the hypothalamic region showed the highest level of expression. Current work includes determining whether stress affects transcript levels of 5-HT<sub>1A</sub> in the toadfish brain and expressing the receptor in *Xenopus laevis* oocytes to provide definitive evidence that the receptor in toadfish is pharmacologically the same as it is in mammals. These studies will help to provide a non-mammalian model for the human 5-HT<sub>1A</sub> receptor in hope of shedding new light on the mechanism of depression.

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**A5.6****11:00 Sunday 28th June 2009****Advancements in understanding the stress physiology of Atlantic cod**

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Stress in an inevitable aspect of sea-cage aquaculture and is known to have several negative impacts on fish, including Atlantic cod (*Gadus morhua*). Therefore, an improved understanding of stress physiology is necessary to better manage and mitigate these negative impacts (e.g. immunosuppression). The Genome Canada-funded Atlantic Cod Genomics and Broodstock Development Project (CGP) has contributed greatly to the study of cod biology through the development of resources such as an extensive EST database ([www.codgene.ca](http://www.codgene.ca)) and a 20,000 gene microarray. Within this project, we have been characterizing molecular pathways and physiological processes involved in the stress response using genomic techniques. For example, we have identified 3258 putative transcripts from cDNA libraries enriched for genes responsive to heat-shock, and validated several of them using QPCR (e.g. HSP90 $\alpha$ , HSP70-1, GRP78, GRP94). We have identified individuals/families with different magnitudes of cortisol response to handling- and heat-stress, and used QPCR to examine if differences in the expression of genes involved in cortisol synthesis (i.e. StAR protein, P450scc and 3 $\beta$ HSD) determine whether a fish is a high or low responder. Furthermore, we are currently characterizing several stress relevant genes such as the glucocorticoid receptor using bi-directional rapid amplification of cDNA ends (RACE) and genome walking. An overview of this work will be presented.

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#### A5.7

11:15 Sunday 28th June 2009

#### Effect of selection for stress responsiveness on neural plasticity: A gene expression study

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In rainbow trout (*Oncorhynchus mykiss*), selection for divergent post-stress plasma cortisol levels has yielded a low (LR) and a high responsive (HR) trout strain. A suite of correlated cognitive, behavioral and physiological differences have been documented in these fish; with proactive LR fish being bolder than and socially dominant over HR fish in most situations. Post-stress plasma concentrations of cortisol are lower in LR fish than in HR fish, and cortisol can degrade neurons and inhibit neurogenesis. Hence, altered neural plasticity could be mediating the divergent behavioral profiles of the HR/LR trout lines. The expression of cortisol receptors and genes involved in neurogenesis and cell proliferation was quantified by real time PCR in brain tissue from stressed (2 $\times$ 2 h of confinement over two consecutive days) and control fish of both lines. Short term stress led to increased expression of Proliferating Cell Nuclear Antigen (PCNA) in the hypothalamus and telencephalon of both HR and LR fish, but there were no line differences. Markers of cell differentiation and survival (BDNF and NeuroD) remained unaffected by both short term stress and selection regime. Brain GR expression was also unaffected by both stress and line. Telencephalon and hypothalamus MR gene expression was higher in non-stressed LR than HR fish, and stress induced a decrease in MR expression in the telencephalon of LR, but not HR fish. Increased forebrain MR expression therefore provides an alternative hypothesis to explain proactive behavior in stress resistant animals.

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#### A5.8

11:30 Sunday 28th June 2009

#### Bioavailability and oxidative stress response in Zebrafish fed <sup>109</sup>Cd enriched *G. pulex* sub-cellular fractions

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Dietary metal uptake in predators may be affected by the sub-cellular distribution of accumulated metals in prey organisms as sub-cellular fractions may have different trophic availabilities. In the present study, the prey species *Gammarus pulex* was exposed to radiolabeled <sup>109</sup>Cd(II) for 13 days. Post-exposure, the insoluble metal-rich granule and exoskeletal fraction (43.02 $\pm$ 1.49%) and the soluble heat stable (MT-like) cytosol (24.76 $\pm$ 1.89%) were the sub-cellular components of greatest metal content ( $p < 0.05$ ). From these <sup>109</sup>Cd(II)-containing fractions food items were produced, bound to gelatin and fed to the model predator *Danio rerio* (Zebrafish) ( $n = 7$ ). For both feed types a bi-phasic depuration pattern was seen. The assimilation efficiency of cadmium bound to the soluble fraction was 32.12 $\pm$ 5.58% which was significantly greater than the assimilation efficiency of insoluble <sup>109</sup>Cd(II) 12.97 $\pm$ 2.11% ( $p < 0.05$ ). At 72 h post feeding, the intestinal tract of each fish was removed and metal content of the gut and carcass in relation to the whole fish was determined. Of the metal retained by the fish, 93.77 $\pm$ 4.87% of soluble <sup>109</sup>Cd(II) had crossed the gut epithelia whereas 45.95 $\pm$ 6.52% of the insoluble <sup>109</sup>Cd(II) remained within the intestine. Lipid peroxidation was measured as TBARS in the dissected guts. Both <sup>109</sup>Cd(II) feeds led to significantly increased TBARS levels compared to the control group (gelatin only), but insoluble feed caused significantly more lipid peroxidation than soluble feed ( $p < 0.01$ ). This study suggests that by remaining in the intestine insoluble metal, which has been previously described as biologically unavailable, is able to cause oxidative injury to the digestive tract of teleost fish.

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#### A5.9

11:45 Sunday 28th June 2009

#### Energetics of PCBs-contaminated juveniles of common sole (*Solea solea* L.): Aerobic metabolism and tolerance to hypoxia

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Increasing pressure of anthropogenic activities in the coastal zones leads to an amplification of environmental fluctuations in aquatic ecosystems (e.g. in oxygen), as well as to the introduction of new elements, such as organic pollutants (e.g. PCB). The aim of our study was to investigate the effects of PCB on the metabolism in juveniles of sole (*Solea solea*), which use coastal ecosystems as nursery. In particular, we tested the hypothesis that exposition to PCB 1) reduce aerobic metabolic scope by interfering with metabolic processes and/or 2) induce supplementary energetic costs related to process of detoxification. Furthermore, interactions between PCB (a mixture of CB153, CB149, CB118 and CB 105) and hypoxia were investigated by assessing the tolerance to hypoxia in contaminated individuals. Experiments were carried out on sole of 0–1 year old. Fish were contaminated *via* food and tested after 30 and 60 days of contamination. Sole were exposed to four treatments: a group was fed with uncontaminated food, a group was fed with food which contained the solvent utilized to coat the PCB; and two groups were fed with food which contains two different concentrations

( $\Sigma$ PCB = 600  $\mu$ g PCB/kg of food; 2100  $\mu$ g PCB/kg of food). Results will be discussed based on the assessment of active metabolic rate, standard metabolic rate and critical oxygen concentration.

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#### A5.10

12:00 Sunday 28th June 2009

##### Involvement of respiratory chain in RVD process in turbot (*Scophthalmus maximus*) hepatocytes

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Regulatory volume decrease (RVD) constitutes a fundamental process that turbot hepatocytes are able to perform when exposed to hypo-osmotic stress. RVD is an integrative mechanism that involves various elements of the cellular machinery. Among others, ATP is an essential protagonist: it is released in extracellular medium after hypo-osmotic shock and acts as an auto/paracrine factor to trigger other signalling events. The origin of this ATP remains unclear and, to our knowledge, no information exists about the potential link between RVD and mitochondrial respiration. In our study, when cells were exposed to respiratory chain inhibitors (KCN 1 mM, rotenone 1  $\mu$ M, antimycin A 50  $\mu$ M, oligomycin 2.5  $\mu$ M or iodoacetic acid 1 mM), significant preventions of RVD process and hypo-osmotic ATP release were observed. Thus, these results indicate that aerobic metabolism could contribute to RVD process in turbot hepatocytes, *via* ATP production. However, our results suggest that mitochondrial respiration play an indirect role in RVD process as hypo-osmotic stimulation of cells did not induce an increase in oxygen consumption. Our hypothesis is that, after production by aerobic metabolism, ATP could be stored in intracellular vesicles until the moment cells need to release it to face hypo-osmotic swelling. Indeed, we have shown that hypo-osmotic ATP release could occur by exocytosis. However, in our study, exocytosis activity was not prevented by respiratory chain inhibitors. Consequently, further experiments, based on vesicular-stores ATP staining, will allow to clarify the role of exocytosis in the process and to precise the sequence of cellular events.

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#### A5.11

12:15 Sunday 28th June 2009

##### Uptake, tissue distribution and excretion of dietary cadmium and copper in discus fish *Symphysodon* spp.

Richard J. Maunder (University of Plymouth), Katherine A. Sloman (University of Plymouth), Adalberto L. Val (Instituto Nacional de Pesquisas de Amazonas, INPA), Janet Pearce (University of Plymouth), Jonathan Buckley (University of Plymouth)

Discus fish, *Symphysodon* spp. are a native cichlid species to the Amazon Basin. They show a highly specialised parental care strategy

where fry feed from the epidermal mucus of both parents for the first few weeks post hatch. This obligate mucus feeding stage makes discus particularly interesting to study in relation to the uptake and elimination of trace metals, which are significant contaminants in areas of their native habitat. Any metal contaminants sequestered by the adults in their epidermal mucus are likely to be passed on as dietary contaminants to their offspring, but nothing is currently known about the response of discus to dietary metals. In this study we investigated the fate of dietary copper (Cu) and cadmium (Cd) in juvenile discus, and also tested the hypothesis that epidermal mucus is involved as an excretory pathway for metals in *Symphysodon*. Groups of fish were exposed to Cu or Cd in the diet (nominal concentration 400 mg kg<sup>-1</sup>, fed at 2% body weight day<sup>-1</sup>) and contaminant levels in the epidermal mucus, plasma, gill, liver, kidney, gut and muscle tissues were determined during a four week exposure. Mucus Cd concentrations were significantly higher than control levels throughout the experiment. Changes in skin histology were also considered in relation to mucus producing cells. The results demonstrate the distribution of metal contaminants when juvenile discus are exposed to a dietary exposure and give an indication of whether metals ingested by breeding wild fish could be rapidly passed onto their young.

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#### A5.12

13:30 Sunday 28th June 2009

##### The effects of metal pollution on the population genetic traits of brown trout (*Salmo trutta* L.) living in the River Hayle, Cornwall, UK

Nicolas R. Bury (King's College London), Christopher Durrant (King's College London)

Brown trout (*Salmo trutta* L.) are one of the most sensitive fish species to metals. However, paradoxically trout can be found in the lotic ecosystems of the South-West of England; a rich metallogenic region whose rivers possess exceptionally high metal loads. One example is the River Hayle catchment area where mining activity ceased in the late 19th century, and whose current waters are toxic to metal naïve brown trout, but possess a vibrant trout population. Severe pollution causes massive mortalities reducing population size leading to genetic bottlenecks and inbreeding that is detrimental to the genetic integrity of isolated populations. This study aimed to identify if the legacy of metal pollution is reflected in the populations genetic structure of trout living in the River Hayle.

Metal influx from disused mine adits divides the river into three sections: "clean" upper section, severely contaminated middle section, and a lower section with raised metals. Trout populations are present above and below the middle region, while this region itself remains void of life. The first hypothesis tested was that the contaminated region presented a barrier to gene flow. However, the population genetics data suggest that genetic information is readily transferred between upper and lower regions. But, results show that there are signs of inbreeding and reduced genetic diversity. The results indicate that brown trout in rivers of the South-West are adapted to elevated metals, but there is genetic diversity cost associated with this adaptation.

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**A5.13****13:50 Sunday 28th June 2009****From *Daphnia* to geckos: The non-genetic component of individual variation**

Katherine A. Sloman (University of Plymouth), Jonas Haag (University of Tasmania), Peter B. Frappell (University of Tasmania)

Parthenogenetic clonal siblings present an interesting model for the study of individual variation. Genotype is essentially identical in these animals and individual differences in behaviour and physiology are a result of either environmental or epigenetic effects. Epigenetic changes may be influenced by environmental conditions but may also occur as stochastic fluctuations in developmental pathways. Here we investigated individual variation in sibling *Daphnia carinata* and the parthenogenetic gecko, *Heteronotia binoei*, with particular interest in thermal preferences. Clonal *Daphnia* siblings from the same brood demonstrated a broad spectrum of thermal preferences suggesting that a single clonal brood would be able to survive in a wide range of environments. Individual variation in thermal preference of *H. binoei* was less apparent. In *H. binoei*, position within a thermal gradient was strongly influenced by social interactions; social effects were absent in *Daphnia*. Our results demonstrate that populations with little to no genetic variability are physiologically and behaviourally diverse with significant implications for population responses to abiotic stressors such as climate change.

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**A5.14****14:10 Sunday 28th June 2009****Does physiological capacity determine dominance in a social hierarchy?**

Elektra Sinclair (University of Sydney), Frank Seebacher (University of Sydney)

Aggressive and submissive behaviour can incur both benefits and costs. Aggressive behaviour enables individuals to assert dominance over others and potentially gain more mating and feeding opportunities. However, these behaviours require considerable energy expenditure and can expose dominant individuals to increased risk of predation due to their lack of defensive behaviour. Male mosquitofish (*Gambusia holbrooki*) establish stable hierarchies, which shows that the benefits of aggressive behaviour are greater than the costs for some fish but not for others. One explanation for this is that the physiological capacities of an individual determine which behavioural strategy will be utilised. For example, if a fish has a fast escape response (C start) and a high sustainable swimming speed (Ucrit), facilitated by high anaerobic and oxidative ATP production capacities, it may be able to initiate and avoid attacks more easily, and sustain longer pursuits than other fish in the group. We tested the stability of *G. holbrooki* dominance hierarchies over time, and we hypothesised that the dominant male in a hierarchy has: (a) faster C starts, (b) higher Ucrit, (c) greater creatine phosphokinase (CP) activity and (d) greater citrate synthase (CS) activity compared to more subordinate individuals within the group.

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**A5.15****14:25 Sunday 28th June 2009****Bi-parental mucus feeding: A unique example of parental care in an Amazonian cichlid**

Jonathan Buckley, (The University of Plymouth) Richard Maunder (The University of Plymouth), Andrew D. Foey (The University of Plymouth), Janet Pearce (The University of Plymouth), Katherine Sloman (The University of Plymouth)

The Amazonian cichlid, *Symphysodon* spp. provides a unique example of parental care in fish due to both the biparental nature of parental care in this species, as well as the ability of *Symphysodon* parents to provide a post hatch form of nutrition via parental mucosal secretions to their offspring. This extensive provision of care, which can last up to a month, imposes a physiological demand on the parents which consequently gives rise to conflict between the parent and offspring. Here, we investigated the conflict between parents and offspring over a breeding cycle by examination of parental and juvenile behaviour. We also examined the content of parental mucosal secretions using a suite of physiological assays (total protein, amino acids, cortisol, IgM and Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup>). Over the course of the breeding cycle a significant increase in offspring bite rate was recorded and was correlated with an increase in the frequency of turns the male and female took at caring for the fry suggesting a gradual increase in conflict throughout the breeding cycle. During this period no significant differences were observed in the amount of time each parent assigned to the care of offspring suggesting that parental duties were equally shared between parents. Variations in the content of parental mucus samples were found throughout the breeding cycle and give insight into the provision of parental care in this species.

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**A5.16****14:40 Sunday 28th June 2009****Diversity in terrestrial food uptake strategies within testudinoids (Chelonia; Cryptodira)**

Nikolay Natchev (Vienna University, Austria), Egon Heiss (Vienna University, Austria), Patrick Lemell (Vienna University, Austria), Josef Weisgram (Vienna University, Austria)

All those recent turtles that can complete the entire feeding process on land belong to a single cryptodiran family – Testudinoidea. Within this group, terrestrial feeding has probably evolved independently several times. As the ancestor of all testudinoids was a purely aquatic animal, one can formulate two differing major hypotheses concerning the food uptake kinematics on land: 1. if the feeding neuromotor systems are conserved during evolution, then the food uptake modes in testudinids (tortoises), emydids and geoemydids will exhibit similar neuromotor patterns; 2. if the feeding processes in testudinoids have an adaptive character, then the kinematics of the feeding apparatus will show higher flexibility. Using high-speed cinematography and high-speed cineradiography, we determined that “lingual food prehension” is typical only for some highly evolved species belonging to the subfamily Testudinidae. Basal testudinids like *Manauria emys emys* and *Rhynoclemmys pulcherrima incisa*, on the other hand, despite possessing large

tongues use obligatory “jaw prehension” for food uptake. Within emydids and geoemydids, only few species are able to feed on land. The kinematical patterns of terrestrial food uptake vary considerably within these groups. Nonetheless, all investigated specimens used “jaw prehension” independent of the food types offered in our experiments. Within testudinoids, the mode of the “initial food contact” seems to bear a phylogenetic signal, because “lingual prehension” has evolved only within the predominantly herbivorous tortoises.

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#### A5.17

14:55 Sunday 28th June 2009

##### Calling heights chosen by male speckled bush crickets (*Leptophyes punctatissima*)

David J. Robinson (Open University), Patricia J. Ash (Open University), Marion J. Hall (Open University)

*Leptophyes punctatissima* is a flightless bush cricket found in the southern part of the UK. The male song is a small number of 1 ms duration syllables at a centre frequency of 40 kHz, to which a female will respond if willing to mate. Her reply must come within a fixed time window of 10 ms, for the male to recognise her. The insects are most active at night. We describe the use of reflective tags to follow their movements in a monitored, natural environment and a system of triangulation using ultrasound detectors to estimate their position in woodland. Unexpectedly, they have been detected singing from high in pine trees, in excess of 10 m above the ground. Surveys of habitats for the newly hatched and early instars show that they feed on plants at ground level and it is likely that the eggs are laid low down, although there are no details known yet of egg laying sites from surveys. We consider the conditions that limit the acoustic interchange between males and female, the significance of the height at which some males call and the possible selective advantages that might favour singing at height.

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#### A5.18

15:40 Sunday 28th June 2009

##### Intestinal $\text{HCO}_3^-$ secretion plays a role in postprandial acid–base balance in gulf toadfish, *Opsanus beta*

Josi R. Taylor (RSMAS, University of Miami, FL, USA), Martin Grosell (RSMAS, University of Miami, FL, USA)

Marine teleost fish possess an elegant array of intestinal transporters allowing them to ingest and process seawater to maintain hydromineral balance in a dehydrating environment. Intestinal  $\text{HCO}_3^-$  secretion by apical  $\text{Cl}^-/\text{HCO}_3^-$  exchange plays an integral role in this osmoregulatory process by conducting  $\text{Cl}^-$  absorption and facilitating precipitation of  $\text{CaCO}_3$  pellets which not only aid water absorption but also contribute significantly to oceanic carbon cycling. In addition, we suggest that intestinal  $\text{HCO}_3^-$  secretion may play a role in whole animal acid–base balance, specifically by alleviating the metabolic alkalosis (“alkaline tide”) commonly experienced during digestion. Evidence of postprandially elevated intestinal  $\text{Cl}^-/\text{HCO}_3^-$  exchange in seawater-acclimated gulf toadfish (*Opsanus beta*) in the absence of alkaline tide inspired a detailed investigation of postprandial changes in  $\text{HCO}_3^-$  secretion, electrophysiology, and  $\text{O}_2$  consumption by the isolated toadfish intestine. Intestinal  $\text{HCO}_3^-$  secretion rates are significantly

elevated during the entire 48 h postprandial period, peaking with a 60.2% increase 3 h post-feeding ( $1.32 \pm 0.02 \mu\text{mol cm}^{-2} \text{h}^{-1}$ ). Postprandial  $\text{O}_2$  consumption rates show that the intestinal epithelium is subject to metabolic rate increase commonly termed specific dynamic action (SDA). Rates of  $\text{O}_2$  consumption are significantly increased between 3 and 12 h post-feeding (Fig. 1), with a 90.2% increase during their peak at 6 h ( $1.02 \pm 0.05 \mu\text{mol cm}^{-2} \text{h}^{-1}$ ). The temporal coordination of these physiological responses to feeding suggests that increases in intestinal  $\text{HCO}_3^-$  secretion precede and likely reduce the inevitable acid–base balance disturbance associated with digestion, rather than increasing secondarily as a consequence of endogenous  $\text{CO}_2$  production attributable to SDA in the intestinal tissue.

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#### A5.19

16:00 Sunday 28th June 2009

##### The regulation of intestinal $\text{HCO}_3^-$ secretion by marine fish: Responding to changes in luminal osmotic pressure

Jonathan Whittamore (University of Exeter), Rod Wilson (University of Exeter), Martin Grosell (University of Miami)

Marine teleost fish are osmoregulators maintaining the composition of their body fluids independent of the seawater environment. To achieve this homeostasis and avoid dehydration they continuously drink the surrounding water. The intestine therefore has a key role, responsible for the absorption of water and excretion of excess divalent ions from imbibed seawater. To help fulfil these functions it secretes bicarbonate ( $\text{HCO}_3^-$ ) into the lumen, producing a characteristically alkaline gut fluid (up to pH 9), which is independent of digestion. Apical  $\text{Cl}^-/\text{HCO}_3^-$  exchange significantly contributes to alkalisation, with intracellular  $\text{CO}_2$  hydration supplying the majority of  $\text{HCO}_3^-$  for secretion, with the necessary removal of acidic equivalents ( $\text{H}^+$ ) across the basolateral membrane. Observations from the killifish (*Fundulus heteroclitus*) intestine *in vitro* have revealed that increasing luminal osmotic pressure results in a rapid reduction in the rate of  $\text{HCO}_3^-$  secretion. The intestine of marine fish is likely to experience large shifts in osmolarity on a regular basis during digestion, as well as coping with the continuous ingestion of seawater. The epithelium is therefore predicted to rely on the rapid recruitment of various ion transport mechanisms to regulate cell volume and luminal osmotic pressure. Using a combination of *in vivo* and *in vitro* experiments, the present study set out to explore the hypothesis that the response of  $\text{HCO}_3^-$  secretion to hyperosmolarity is brought about by a shift in  $\text{H}^+$  secretion, from basolateral to apical, which in combination with an extracellular carbonic anhydrase will dehydrate luminal  $\text{HCO}_3^-$  thus facilitating a reduction in luminal osmotic pressure.

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#### A5.20

16:15 Sunday 28th June 2009

##### What limits tolerance to hypersalinity in marine teleosts?

Janet Genz (University of Miami, RSMAS), Martin Grosell (University of Miami, RSMAS)

Marine teleost fish maintain water balance via absorption of ingested monovalent ions and water across the intestinal epithelium. Subsequent

extrusion of the absorbed ions across the gill results in net water gain. As water is absorbed, relatively impermeant  $Mg^{2+}$  and  $SO_4^{2-}$  ions are concentrated within the intestinal lumen. The present study assesses the osmoregulatory implications of accumulated  $MgSO_4$  in the luminal fluid for NaCl and water absorption by the intestinal epithelium. Toadfish (*Opsanus beta*) were exposed to hypersaline seawater (SW) (50, 60, 70, 77 and 85 ppt), and also to seawater adjusted from 35 ppt to an osmolality equivalent to SW by the addition of NaCl only (NaCl-adjusted seawater, NASW). NASW thus had lower  $[MgSO_4]$  and higher  $[NaCl]$  than SW at all salinities. Blood plasma and intestinal fluid were sampled after 24 h in these media, and osmolality, total  $CO_2$ , and ion composition were measured. Both intestinal fluids and plasma of fish in SW had significantly higher osmolality than fish exposed to NASW at 70 and 77 ppt. Plasma  $Mg^{2+}$  was lower in fish in NASW than in SW at all salinities; the same was true for luminal  $Mg^{2+}$  and  $SO_4^{2-}$ . All toadfish exposed to 85 ppt SW expired within 24 h, whereas less mortality occurred in NASW-exposed fish. This suggests that  $[MgSO_4]$  in intestinal fluids, rather than NaCl and osmotic gradients between the extracellular and luminal fluids, plays a key role in limiting hypersalinity tolerance in the marine teleost *Opsanus beta*. Supported by NSF awards 0416440, 0714024, and 0743903.

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#### A5.21

16:30 Sunday 28th June 2009

#### Calcium metabolism in a freshwater cartilaginous fish, the lake sturgeon, *Acipenser fulvescens*

Peter J. Allen (University of Mississippi), Dirk Weihrauch (University of Manitoba), Stephan J. Peake (University of New Brunswick), Gary Anderson (University of Manitoba)

Calcium regulation in vertebrates is achieved through the coordination of renal and extrarenal routes. Terrestrial vertebrates balance calcium metabolism through dietary intake with renal output, in addition to utilization of bony tissue as an internal calcium store. In teleost fish not only is bony tissue (skeleton and scales) utilized, but the environment also represents a significant calcium store, and the gills, along with intestinal and renal tissue are actively involved in regulating internal calcium concentrations. With the lack of bony tissue in cartilaginous fishes, internal calcium stores are somewhat limited, and these limitations are particularly challenging in fishes inhabiting FW. In the present study, juvenile lake sturgeon *Acipenser fulvescens*, a FW cartilaginous fish, were acclimated to hypo- (0.1 mM), normo- (0.4 mM) or hyper-calcaemic (3.3 mM) environments. Calcium influx and efflux were measured to provide net flux which was found to be inward directed in all treatments in the following order: hypo > normo > hyper-calcaemic. Drinking rates followed a similar trend suggesting a role for the intestine in calcium regulation. This was supported by the accumulation of  $^{45}Ca$  in the gastrointestinal tract measuring  $31.3 \pm 1.9$ ,  $21.7 \pm 1.5$  and 0 nmol/h/g in hypo-, normo and hyper-calcaemic adapted fish. In a subsequent experiment, gill, pyloric caecae, mid-intestine, posterior intestine and renal tissue were sampled in each treatment group to examine the role of the epithelial calcium channel (ECaC) in overall calcium metabolism. Results will be discussed in relation to mRNA expression of ECaC and the role of the various tissues in calcium regulation in the lake sturgeon.

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#### A5.22

16:45 Sunday 28th June 2009

#### A role for CHH in the regulation of kidney-like function in freshwater land-crabs: A study of the Christmas Island blue crab, *Discoplax hirtipes*

Lucy M. Turner (University of Bristol), Simon G. Webster (Bangor University), Steve Morris (University of Bristol)

The gecarcinid land crab *Discoplax hirtipes* is distributed throughout the tropical Indo-Pacific and exhibits a dichotomous distribution depending on rainfall. In the dry season these crabs are restricted to areas of freshwater seepages, however, once the wet season begins the crabs disperse over a wider area. This species has evolved to a terrestrial-amphibious existence with adaptations including the development of lungs and the utilisation of the gills as a kidney analogue to reclaim urinary salts. The ecology is dependent on the seasonally variable rainfall and is accompanied by important changes in underlying mechanisms of ionic and osmotic regulation. Neuropeptides of the Crustacean Hyperglycaemic Hormone family (CHH) were initially identified as important in carbohydrate metabolism but have recently been implicated in controlling osmoregulation. Gecarcinids produce relatively large amounts of CHH and this hormone is a prime contender for regulating the seasonally variable physiological state and specifically salt balance in *D. hirtipes*. Molecular work shows two forms of CHH to be present in *D. hirtipes* sharing a common central amino acid sequence (hitherto identified in few crustacean species). Bioassays have confirmed the hyperglycaemic response to both forms and we are investigating the role these play in osmoregulation. Field (Christmas Island, Indian Ocean) and laboratory studies of gill function as a kidney analogue (unidirectional ion influx ( $^{22}Na$  tracer) and branchial pumps ( $Na^+/K^+$ -ATPase activity)) have revealed the seasonal specific roles of each of these CHHs on osmoregulation in *D. hirtipes*.

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#### A5.23

10:30 Monday 29th June 2009

#### Heat transfer in transections of thermoregulating bigeye tuna (*Thunnus obesus*)

Jess Boye (Zoophysiology Dept. of Biological Sciences, Aarhus University), Michael Musyl (JIMAR, University of Hawaii), Richard Brill (Virginia Institute of Marine Science), Hans Malte (Zoophysiology Dept. of Biological Sciences, Aarhus University)

We developed a two dimensional heat flux model to elucidate routes and rates of heat transfer within bigeye tuna in both the steady-state and time-dependent settings. In modeling the former situation, we adjusted the efficiencies of heat conservation in the red and the white muscle so as to make the output of the model agree as closely as possible with observed cross-sectional isotherms. In modeling the latter situation, we applied the heat exchanger efficiencies from the steady state model to predict the distribution of temperature and heat fluxes in bigeye tuna during their extensive daily vertical excursions. The simulations yielded a close match to the data recorded in free swimming fish and strongly point to the importance of the heat producing and heat conserving properties of the white muscle. The best correspondence between model output and observed data was obtained when the counter current heat exchangers in the blood flow pathways to the red and white muscle

retain 99% and 96% (respectively) of the heat produced in these tissues. Our model confirms that the ability of bigeye tuna to maintain elevated muscle temperatures during their extensive daily vertical movements depends on their ability to rapidly modulate heating and cooling rates. This study shows that the differential cooling and heating rates could be fully accounted for by a mechanism where blood flow to the swimming muscles was either exclusively through the heat exchangers or completely shunted around them, depending on the ambient temperature relative to the body temperature. Our results therefore strongly suggest that such a mechanism is involved in extensive physiological thermoregulatory abilities of endothermic bigeye tunas.

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#### A5.24

10:50 Monday 29th June 2009

#### Control of breathing and respiratory gas transfer in goldfish (*Carassius auratus*) experiencing gill remodeling

Steve F. Perry (University of Ottawa), Velislava Tzaneva (University of Ottawa), Kathleen M. Gilmour (University of Ottawa)

Goldfish, like crucian carp, can drastically alter functional lamellar surface area by producing or retracting an interlamellar cell mass (ILCM). By doing so, they are able to match functional surface area to metabolic or energetic requirements and thus limit passive diffusive movements of salt and water across the gill. The impact of such changes in lamellar surface area on respiratory gas transfer have not been examined in any detail and thus a major goal of the present study was to assess the consequences of gill remodeling on blood gases in goldfish at 25 °C (no ILCM and thus large functional surface area) and 7 °C (fully formed ILCM and thus reduced functional surface area). In accordance with theory, the results demonstrated significant impairment of CO<sub>2</sub> excretion with little impact on branchial O<sub>2</sub> transfer. The capacity of fish with an ILCM to sustain efficient O<sub>2</sub> transfer (as estimated from PaO<sub>2</sub> measurements), especially under conditions of hypoxia, was remarkable given the extent of the surface area reduction.

An additional consequence of the formation of an ILCM is the covering of lamellar neuroepithelial cells (NECs), which are believed to be O<sub>2</sub> chemoreceptors. Thus, a second goal of the current study was to assess the ability of fish with an ILCM to respond to ventilatory stimuli (hypoxia, sodium cyanide and hypercapnia). The results demonstrated that fish with an ILCM did not exhibit any impairment of chemoreception which we attribute to a re-distribution of the NECs from the lamellar epithelium to the outer edge of the ILCM.

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#### A5.25

11:10 Monday 29th June 2009

#### Cellular investigation into the negative-force frequency response in ventricular myocytes from the varanid lizard

Daniel E. Warren (University of Manchester), Gina L. Galli (University of British Columbia), Simom Patrick (University of Manchester), Holly A. Shiels (University of Manchester)

Varanid lizards (*Varanus exanthematicus*) have higher heart rates, cardiac outputs and blood pressures than the average ectotherm, with values approaching those of mammals. Isometric force developed by isolated cardiac muscle from varanids decreases as frequency increases – a negative force–frequency response. To investigate the cellular mechanisms underlying the negative force–frequency relationship in the ventricle of the varanid lizard we measured cell and sarcomere shortening, intracellular Ca<sup>2+</sup>, action potentials and K<sup>+</sup> currents in isolated ventricular myocytes at 0.2–1.0 Hz. As stimulation frequency increased, time to peak shortening, diastolic length, percent change in sarcomere length and relaxation time all decreased significantly. This corresponded to decreases in time to peak Ca<sup>2+</sup> and Ca<sup>2+</sup> transient amplitude and a 6-fold increase in diastolic Ca<sup>2+</sup> concentrations. The time constant for the decay of the Ca<sup>2+</sup> transient ( $\tau$ ) decreased at lower frequencies, but then plateaued at moderate frequencies and did not change above 0.5 Hz. Action potential duration decreased with increased frequency but the currents responsible remain elusive. We conclude that varanid ventricular myocytes reach an upper limit of Ca<sup>2+</sup> extrusion that is likely responsible for the large increase in diastolic [Ca<sup>2+</sup>]<sub>i</sub>. We hypothesize that this high diastolic Ca<sup>2+</sup> inactivates L-type Ca<sup>2+</sup> channels and/or ryanodine Ca<sup>2+</sup> release channels, leading to decreased Ca<sup>2+</sup> transient amplitude and sarcomere shortening.

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#### A5.26

11:30 Monday 29th June 2009

#### Specialisations in excitation–contraction coupling contribute to high cardiovascular performance in varanid lizards

Gina L. Galli (The University of British Columbia), Daniel Warren (The University of Manchester), Holly A. Shiels (The University of Manchester)

For the vast majority of ectotherms, cardiovascular performance is several folds lower than a comparably sized mammal or bird at their physiological temperature. However, varanid lizards have higher heart rates, cardiac outputs and blood pressures than the average ectotherm, with values approaching those of mammals. Specialisations in excitation–contraction (E–C) coupling may contribute to the varanids superior cardiovascular performance. We used electrophysiology and fluorescent microscopy to test the hypothesis that Ca<sup>2+</sup> cycling through the L-type Ca<sup>2+</sup> channel, the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger (NCX) and the sarcoplasmic reticulum (SR) is enhanced in varanid lizard myocytes when compared to a more sedentary ectotherm, the turtle. Confocal microscopy was used to visualize and measure ventricular myocyte dimensions. Myocytes were found to be spindle shaped, lacking T-tubules, with a small cell volume (2 pL). The density and kinetics of the L-type Ca<sup>2+</sup> channel current ( $I_{Ca^{2+}}$ ) was similar to other ectotherms, while the reverse mode NCX current ( $I_{NCX}$ ) was 2–3 fold higher than turtle  $I_{NCX}$ . Pharmacological inhibition of the SR led to a 40% reduction in the amplitude of the varanid Ca<sup>2+</sup> transient ( $[Ca^{2+}]_i$ ), and slowed the time course of  $[Ca^{2+}]_i$  decay. This is in stark contrast to turtle  $[Ca^{2+}]_i$  which is insensitive to SR inhibition. In aggregate, our results suggest varanids have an enhanced capacity to transport Ca<sup>2+</sup> through the NCX and SR suggesting specialisations in E–C coupling may provide a means to support high cardiovascular performance.

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**A5.27****11:45 Monday 29th June 2009****Comparison of length-dependent Ca<sup>2+</sup> activation of cardiac myofilaments between the rat and the rainbow trout**

Simon M. Patrick (The University of Manchester), Holly J. Shiels (The University of Manchester), Edward White (The University of Leeds), Olivier Cazorla (Inserm U-637)

Most fish regulate cardiac output via changes in stroke volume whereas most mammals regulate cardiac output via changes in heart rate. We hypothesized that this change in mechanism of regulation would coincide with a change in the myocardial response to stretch. This possibility was tested in permeabilized cardiomyocytes prepared from rat and rainbow trout ventricles, where both sarcomere length (SL) and degree of Ca<sup>2+</sup> activation could be controlled. Myofilament Ca<sup>2+</sup> sensitivity activation was higher in trout than in rat at each SL tested (2.0, 2.3, 2.5 and 2.7 μm). We also found that permeabilized trout myocytes produce greater passive tension at any given SL than rat. This was surprising as the trout heart is known to be more compliant. Interestingly, addition of phosphatase inhibitors in the permeabilizing solution reduced passive tension in the trout cells suggesting that phosphorylation of titin may be important in determining passive tension in particular in trout heart. In conclusion contractile properties in trout seem highly sensitive to the length-dependent modulation.

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**A5.28****12:00 Monday 29th June 2009****Regulation of cardiac metabolic supply and demand during hypoxia in the tilapia (*Oreochromis niloticus*)**

Ben Speers-Roesch (University of British Columbia), Erik Sandblom (Göteborg University), Anthony P. Farrell (University of British Columbia), Jeffrey G. Richards (University of British Columbia)

The ability to balance cardiac ATP supply with demand and down-regulate ATP turnover is vital for the survival of hypoxia-tolerant species during oxygen lack. We investigated the regulation of ATP supply and demand during hypoxia exposure in the heart of tilapia to assess the relative importance of control of these pathways in achieving ATP balance and metabolic depression during hypoxia. During a 24-h hypoxic exposure, ATP levels remained constant in the heart while free ADP and free AMP levels decreased. A decrease in ATP supply was evident from a large, rapid (in < 1 h) decrease in circulating levels of free fatty acids and a modest decrease in activity of pyruvate dehydrogenase (PDH), potentially mediated by PDH kinase. Gene expression analysis and metabolite levels suggested that fatty acid oxidation was not downregulated at the cellular level. On the other hand, measurements of cardiovascular function during graded and prolonged hypoxia showed that cardiac output and cardiac power output (analogous to ATP demand of the heart) decreased rapidly along with whole animal metabolic depression and remained depressed by 50–60% throughout the 8-h hypoxic period. The rapidity and magnitude of the decrease in cardiac work vs. the more modest changes in ATP supply pathways suggests that downregulation of ATP demand is more important in decreasing ATP turnover during metabolic rate suppression in the heart of tilapia during hypoxia exposure.

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**A5.29****12:15 Monday 29th June 2009****Crucian carp heart performance during anoxia and acidosis**

Jonathan A. Stecyk (University of Oslo), Bent C. Larsen (University of Oslo), Göran E. Nilsson (University of Oslo)

The crucian carp (*Carassius carassius*) seems unique among vertebrates in its ability to maintain cardiac performance during prolonged anoxia. We have shown that cardiac output remains unchanged for 5 days of anoxia<sup>1</sup>. We investigated the hypothesis that this phenomenon is possible because the heart likely never experiences an extracellular pH below 7.4 due to the fish's exclusive trait of converting lactate and H<sup>+</sup> ions to ethanol. Performance of isolated, spontaneously contracting heart preparations from cold-acclimated (6–8 °C) crucian carp was measured during exposure to 100 min of: 1) normoxia at pH 7.8; 2) anoxia at pH 7.8; 3) graded acidosis under both normoxia and anoxia (pH decreased by 0.2 units every 20 min from pH 7.8 to 7.0); and 4) graded acidosis during anoxia to a maintained extracellular pH of 7.4. All experiments were conducted at 6.5 °C. As expected, the results revealed that anoxic performance of the crucian carp heart is severely impaired by a fall in pH below the normal anoxic level of 7.4. Heart rate, contraction kinetics and pumping capacity (an estimate of cardiac output obtained by multiplying heart rate and maximal contractile force<sup>2</sup> of anoxic hearts exposed to pH below 7.4 were significantly decreased compared to anoxic hearts exposed to or above pH 7.4. These findings suggest that in nature, heart performance may not be able to meet the needs of the body if blood pH is not maintained above 7.4.

1. Stecyk et al., 2004. *Science* 306,77.

2. Matikainen and Vornanen 1992. *J. Exp. Biol.* 167, 203–220.

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**A5.30****13:30 Monday 29th June 2009****Molecular evolution of myoglobin in small diving mammals**

Scott Mirceta (Liverpool University), Kevin L. Campbell (University of Manitoba), Michael Berenbrink (Liverpool University)

A consistent hallmark of the most accomplished marine mammalian divers is the elevated concentration of oxygen-storing myoglobin (Mb) in locomotory muscles. In our ongoing studies we have found distinct molecular signatures of Mb amino acid sequences in whales and seals, which indicate a convergent evolutionary trend towards an increase in acid–base relevant histidine residues and overall net positive charge of the protein. We interpret these trends as adaptations for buffering lactic acid that accumulates during long dives, and for increased Mb solubility. Here we investigate whether these trends are also apparent in the Mb of small aquatic mammals that dive for considerably shorter durations than seals and whales. Myoglobin sequences were obtained using standard molecular biology techniques and from public databases. Within rodents we examined beaver and muskrat, which independently evolved semi-aquatic lifestyles and diving behaviours. In both cases the number of acid–base relevant Mb histidines increased from 2 to 5, when compared to their closest terrestrial relatives. In addition, Mb net charge amounted to about twice the value found in terrestrial rodents. Among insectivores, Mb histidine content did not show any obvious trend. However, the semi-aquatic star-nosed mole and water shrew both showed distinctly

elevated Mb net charge compared to their close terrestrial relatives, the coast mole and short-tailed shrew. Our results surprisingly suggest convergent increases in net positive charge in Mb across all diving mammals from water shrew to sperm whale.

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#### A5.31

13:45 Monday 29th June 2009

#### **H<sub>2</sub>S mediates the hypoxic vasoconstriction in trout gills through a production of reactive oxygen species**

Nini Skovgaard (Department of Pharmacology, Aarhus University), Kenneth R. Olson (Indiana University School of Medicine-outh Bend, IN)

Hypoxic pulmonary vasoconstriction (HPV) is an adaptive response that diverts pulmonary blood flow from poorly ventilated and hypoxic areas of the lung to more well-ventilated parts. This response is important for the local matching of blood perfusion to ventilation and improves pulmonary gas exchange efficiency. HPV is an ancient and highly conserved response, expressed in the respiratory organs of all vertebrates, including lungs of mammals, birds and reptiles, amphibian skin and fish gills. The mechanism underlying HPV and how cells sense low PO<sub>2</sub> remains elusive. In perfused trout gills (*Oncorhynchus mykiss*) acute hypoxia and H<sub>2</sub>S caused an initial constriction of the vasculature followed by dilation. Inhibition of the enzymes CSE and CBS, which blocks H<sub>2</sub>S production, abolished the hypoxic response. Blockers of all four complexes in the electron transport chain abolished both the hypoxic and the H<sub>2</sub>S mediated constriction. Glutathione, an antioxidant and scavenger of superoxide, attenuated the vasoconstriction in response to hypoxia and H<sub>2</sub>S. Furthermore, an inhibitor of superoxide dismutase attenuated the hypoxic and H<sub>2</sub>S constriction. This strongly indicates that H<sub>2</sub>S mediates the hypoxic vasoconstriction in trout gills. H<sub>2</sub>S may stimulate the mitochondrial production of superoxide, which is then converted to H<sub>2</sub>O<sub>2</sub>. Thus, H<sub>2</sub>O<sub>2</sub> may act as the 'down stream' signalling molecule in hypoxic vascular constriction.

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#### A5.32

14:00 Monday 29th June 2009

#### **Swimming endurance and reactive oxygen species in silver eel red muscle**

Hélène Mortelette (ORPHY-UBO), Christine Moisan (ORPHY-UBO), Philippe Sébert (ORPHY-UBO), Aline Amérand (ORPHY-UBO)

The European eels, *Anguilla anguilla* L. have a particular life cycle. At silver sexually mature stage, they must migrate over 6000 km for their reproduction. The sustained swimming activity during this spawning migration is performed by slow aerobic red muscle which is known to have high degree of phenotypic plasticity in response to energetic requirement and environmental factors. In this context, the relationships between the reactive oxygen species (ROS) metabolism, mainly linked to the mitochondrial activity, and the aerobic swimming performance are studied. Sedentary (control) and trained female silver eels were used. Critical swimming speed (CSS) was determined before the training protocol which consisted in 4 days swimming (each day 10 h at 70% and 14 h at 50% of CSS) then after this swimming period. This training increased significantly the CSS of trained eel for about 6%.

After the training protocol, the *in vitro* oxygen consumption and ROS production in red permeabilised fibres tended to be higher in trained eels than in sedentary ones when measured in maximal conditions of mitochondria functioning. In parallel, antioxidant enzyme activities (superoxide dismutase (SOD), catalase and glutathione peroxidase) and malondialdehyde (MDA) content (lipoperoxidation index) were lower in red muscle of trained eels than in sedentary eels (significant for SOD and MDA). These biochemical changes show that the endurance training may improve maximal capacity of the red muscle oxygen consumption without deleterious effect of ROS. These regulations might participate to the swimming performance efficiency in eel.

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#### A5.33

14:15 Monday 29th June 2009

#### **High hydrostatic pressure improves the swimming efficiency of European migrating silver eel**

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To reproduce, European eels must undergo a long migration without feeding from their freshwater habitats to the Sargasso Sea. During this Atlantic migration, they have to cope with many changes in environmental factors, one of them being hydrostatic pressure. We focus on the effects of hydrostatic pressure on swimming energetics: what are the eventual effects of pressure exposure on swimming efficiency? We have designed a special Blazka type swimming tunnel able to work under pressure. This tunnel was connected to our high pressure water circulation system and placed in the hyperbaric chamber. From the decrease of water oxygen content (Cw<sub>O<sub>2</sub></sub>), oxygen consumption was measured during 30 min in migrating male silver eels (body mass, 86.5 ± 6.2 g) at different swimming speeds (from 0.2 to 1.0 body length (BL)/second (s) by 0.1 BL/s step) first at atmospheric pressure then at 10.1 MPa. Experiments were performed at 14.7 ± 0.2 °C. The results show that pressure increases the energetic swimming efficiency by decreasing oxygen consumption for a given swimming speed. Such a pressure effect could represent a remarkable adaptation enabling eels to spare their energy stores and swim for a long time.

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#### A5.34

14:30 Monday 29th June 2009

#### **Correlations between aerobic and anaerobic performance in individual European sea bass**

Stefano Marras (Université Montpellier 2), Shaun S. Killen (Université Montpellier 2), Guy Claireaux (Université de Bretagne Occidentale), Paolo Domenici (CNR-AMC, Oristano), David J. McKenzie (CNRS, Montpellier)

We investigated the relationships among traits of swimming performance and aerobic metabolic capacity in an experimental population of sea bass. Maximum sustained aerobic and maximum burst anaerobic swimming speeds were measured with a constant acceleration test (CAT) in a swim flume. Maximum aerobic speed was taken as the

point of gait transition from steady swimming to burst-and-coast swimming during the CAT, as determined by video analysis. Aerobic scope was estimated as the difference between excess post-exercise oxygen consumption (EPOC) after the CAT and routine metabolic rate 24 h later, and rate of recovery from EPOC was also taken as an index of individual metabolic capacity. Anaerobic sprint performance was measured in a custom-designed chamber. The C-start escape response was measured by high-speed video. These elements of aerobic and anaerobic swimming performance were analyzed for correlations. Two working hypotheses were investigated. The first was that all of the variables exhibited a similar degree of diversity (coefficient of variation) within the population, and that the variables were all positively correlated. That is, the population comprises good athletes and bad athletes. According to the second hypothesis, certain variables (particularly aerobic and anaerobic performance) may be inversely related as a result of a trade off in swimming modes that use different type of musculature.

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#### A5.35

14:45 Monday 29th June 2009

#### The effects of compensatory growth and reproduction on locomotor performance

Who Seung Lee (University of Glasgow), Pat Monaghan (University of Glasgow), Neil Metcalfe (University of Glasgow)

The adult phenotype can be affected by environmental circumstances in early life. In particular, it has been shown that accelerated growth (prompted by an improvement in environmental conditions after a period of adversity) may subsequently affect a range of fitness traits, including locomotor performance. Here we investigate how accelerated growth affects future swimming performance in three-spined sticklebacks. Growth was manipulated by temporarily holding fish at three different temperatures (high, intermediate, and low) for 4 weeks during the juvenile period. This induced compensatory growth once all fish were returned to a common temperature, such that the mean size of fish in the three treatment groups gradually converged. We measured swimming endurance twice: 1) when the fish from the different manipulation groups had reached the same mean size, and 2) at the end of the subsequent breeding season. Swimming endurance prior to the breeding season did not differ between treatment groups. However, after breeding, endurance was significantly reduced (and fish took longer to recover after exhaustion) in the low temperature group. Since fish in this group had experienced the fastest growth (in compensation for their earlier slow growth at the time of the temperature manipulation), this suggests that rapid growth impairs locomotor performance, but the effects may not be seen until much later in life when the animal is under more severe physiological stress (in this case due to breeding).

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#### A5.36

15:30 Monday 29th June 2009

#### High resting lipoprotein flux of rainbow trout exceeds the energy requirements of endurance swimming

Leonardo J. Magnoni (University of Ottawa), Eric Vaillancourt (University of Ottawa), Jean-Michel Weber (University of Ottawa)

Fish may use lipoproteins instead of albumin-bound fatty acids to fuel endurance exercise, but lipoprotein kinetics have never been measured in ectotherms. *In vivo* bolus injections of labeled very-low-density lipoproteins (<sup>3</sup>H-VLDL labeled *in vivo* from donor fish) and continuous infusions of Intralipid (<sup>3</sup>H-labeled artificial emulsion) were used to investigate the effects of prolonged exercise (6 h at 1.5 body length/s) and heparin (600 U/kg) on the turnover rate of circulating triacylglycerol (TAG) in rainbow trout. We hypothesized that swimming would stimulate TAG turnover rate to fuel working muscles and that heparin would reduce flux by releasing lipoprotein lipase (LPL) from endothelial cells. Results from both tracer methods show that the baseline TAG turnover rate of trout ranges from 24 to 49  $\mu\text{mol TAG kg}^{-1} \text{min}^{-1}$  and exceeds all values measured to date in endotherms. More importantly, this high resting turnover rate is not stimulated during swimming, because it can already cover several times the energy requirements of locomotion. The fact that heparin causes a 50% decrease in baseline TAG turnover rate suggests that fish LPL must be bound to the endothelium for normal tissue uptake of fatty acids supplied by lipoproteins, as in mammals. We propose that the high resting TAG turnover rate of rainbow trout could be needed by ectotherms for rapid restructuring of membrane phospholipids. The continuous tracer infusion method implemented here could be a versatile tool to investigate the potential role of lipoproteins in providing fatty acids for rapid homeoviscous adaptation.

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#### A5.37

15:45 Monday 29th June 2009

#### Mice run faster on a high *n*–6 polyunsaturated fatty acid diet

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A recent review found a strong positive relationship among mammal species between mass-independent variation in burst (or maximum) running speed and the relative content of *n*–6 polyunsaturated fatty acids (PUFAs) in their leg muscles. It was unknown, however, whether this association might also be apparent within a species. We used a crossover design to test for a difference in maximum running speed within 29 individual wild-type mice after two weeks on a diet containing either 10% sunflower (high *n*–6) or linseed (high *n*–3) oil. When fed the sunflower oil diet, mice ran 0.19 m/s faster, on average, than when fed the linseed oil diet (paired one-tail *t*-test:  $P < 0.01$ ). A likely mechanism explaining this result is the known positive effect of a relatively high content of *n*–6 PUFAs on the activity of calcium pumps (SERCA) embedded in the sarcoplasmic reticulum of muscle cells. This would reduce muscle relaxation time and allow faster limb cycling and a faster speed during anaerobic bursts of sprinting. The 6.3% effect size of the diet treatments represents a 0.4 m advantage over a 2 s sprint by a mouse escaping from a predator. In other terms, it matches the 0.6 s improvement in world record times of the men's 100 m sprint between the 1932 and 2008 Olympic Games. Thus, our data support the hypothesis that variation in dietary intake of *n*–6 PUFAs can have a biologically meaningful effect on an individual's maximum running speed.

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**A5.38****16:00 Monday 29th June 2009****Flight speeds of swifts (*Apus apus*): Seasonal differences smaller than expected**

Per Henningsson (Lund University), Håkan Karlsson (Lund University), Johan Bäckman (Lund University), Thomas Alerstam (Lund University), Anders Hedenström (Lund University)

We have studied the nocturnal flight behaviour of the common swift (*Apus apus* L.), by the use of a tracking radar. Birds were tracked from Lund University in southern Sweden during spring migration, summer roosting flights and autumn migration. Flight speeds were compared with predictions from flight-mechanical and optimal migration theory. During spring flight speeds were predicted to be higher than during both summer and autumn due to time restriction. In such cases birds fly at a flight speed that maximizes the overall speed of migration. For summer roosting flights, speeds were predicted to be lower than during both spring and autumn since the predicted flight speed is the minimum-power-speed that involves the lowest energy consumption per unit time. During autumn we expected flight speeds to be higher than during summer but lower than during spring since the expected flight speed is the maximum-range-speed, that involves the lowest energy consumption per unit distance. Flight speeds during spring were indeed higher than during both summer and autumn which indicates time selected spring migration. Speeds during autumn migration were very similar to those recorded during summer roosting flights. The general result shows that swifts change their flight speed between different flight behaviours to a smaller extent than expected. Furthermore, the difference between flight speeds during migration and roosting among swifts was found to be less pronounced than previously recorded.

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**A5.39****16:15 Monday 29th June 2009****Burrowing frog metabolism: Maximising energy savings during dormancy**

Sara M. Kayes (School of Biological Sciences, The University of Queensland), Rebecca L. Cramp (School of Biological Sciences, The University of Queensland), Nicholas J. Hudson (Livestock Industries, CSIRO), Craig E. Franklin (School of Biological Sciences, The University of Queensland)

During dormancy energy conservation is a key priority and as such animals undergo a major metabolic depression to conserve their limited endogenous fuel supplies. Burrowing frogs are exceptional amongst vertebrates that undergo dormancy in that they can survive for several years in the absence of food and water, considerably longer than the most proficient mammalian hibernators. Mitochondrial coupling efficiency, the efficiency at which mitochondria converts oxygen into ATP, significantly affects aerobic metabolism and might be expected to increase during dormancy. Previous studies have shown coupling efficiency to be maintained or even to decrease, however we hypothesised that in order to maximise energy savings during dormancy coupling efficiency would increase in the burrowing frog, *Cyclorana alboguttata*. In this study we measured changes in mitochondrial oxygen consumption during both state 3 and state 4

respiration to examine the effect dormancy had on mitochondrial coupling efficiency in this frog. Our results suggest that, unlike other dormant animals, the frogs can increase mitochondrial coupling efficiency during dormancy, which may help explain the superior lengths of dormancy documented in these frogs compared to hibernators.

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**A5.40****16:30 Monday 29th June 2009****Gene expression in anoxic turtle brain**

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Fresh water turtles (*Trachemys scripta*) have evolved an anoxia tolerance in response to overwintering, buried in anoxic mud at the bottom of lakes. In the absence of oxygen, turtles show severe metabolic depression (90%). In the brain, electric activity is suppressed to decrease ATP turnover leading to comatose. This neuroprotective response involves regulation of GABAergic (inhibitory neurotransmission) and glutamatergic (excitatory neurotransmission) activity to reduce neuronal excitability. In order to investigate the involvement of gene expression in the glutamatergic pathway, we used real-time PCR to assess the effect of anoxia (1 day, 14 days) and temperature (5 °C, 21 °C) on the expression of 18 genes including those coding for 7 N-methyl-D-aspartate (NMDA) receptor subunits (GluN1, 2A-D, 3A-B) and 4 3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor subunits (GluA1-4) (both are glutamate receptors), 2 glutamate transporters (EAAT2-3), 1 activity-regulated cytoskeleton-associated protein (ARC) and 4 proteins involved in GluN-mediated neuroplasticity (the brain derived neurotrophic factor BDNF, the BDNF receptor TrkB1-2, and the cAMP responsive element binding protein 1 CREB1). We used an external standard for normalization.

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**A5.41****16:45 Monday 29th June 2009****Rhinoceros beetles modulate spiracular opening to regulate haemolymph pH**

Philip G. Matthews (The University of Queensland), Craig R. White (The University of Queensland)

Insects are the most diverse and numerous terrestrial air-breathing animals, yet a comprehensive understanding of their respiratory physiology remains elusive. This is clearly seen in the debate surrounding the origins and function of discontinuous gas exchange cycles (DGCs) displayed by many insects at rest. While many hypotheses have been proposed to explain the function of DGCs, no general underlying mechanism driving patterns of insect ventilation has been universally accepted. In the present study, we simultaneously measured *in vivo* pH fluctuations and patterns of CO<sub>2</sub> emission in rhinoceros beetles *Xylotrupes ulysse*. Beetles were restrained within a flow-through respirometry

chamber with a fibre optic pH probe implanted in the haemolymph through the dorsal surface of their thorax. DGCs caused haemolymph pH to fluctuate by 0.1 units, with internal pH declining while the spiracles were closed and rapidly climbing when the spiracles opened. Exposure to hypoxia (5% oxygen in nitrogen) caused the spiracles to open widely and elicited a large burst of CO<sub>2</sub>, causing haemolymph pH to increase from 7.0 to 7.3. If the spiracles were held open by continued exposure to hypoxia, the pH remained high. Once normoxia was restored, the beetles held their spiracles shut, and only resumed normal opening behaviour once internal pH declined to pre-hypoxia levels. The protracted breath hold following hypoxic exposure indicates that the beetles preferentially retain CO<sub>2</sub> in order to restore their haemolymph pH. We therefore propose that insects modulate gas exchange to regulate internal pH, and that DGCs represent an extreme example of this strategy.

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#### A5.42

##### Poster Session – Monday 29th June 2009

##### The basis of vagal efferent control of cardiorespiratory interactions in the Neotropical fish, the pacu, *Piaractus mesopotamicus*

Cléo A.C. Leite (UNESP-C), Edwin W. Taylor (University of Birmingham), Luiz H. Florindo (UNESP-P), Francisco T. Rantin (UFSCar)

In the neotropical fish, the pacu (*Piaractus mesopotamicus*) during progressive hypoxia (before the hypoxic bradycardia) fH initially increased, establishing a 1:1 fH:R ratio. Both a normoxic inhibitory tonus on the heart and the fH adjustments during progressive hypoxia were abolished by atropine injection. Efferent electrical activity in the cardiac vagus (cX) included bursts of activity related to sporadic coughs or changes in ventilation amplitude that caused a decrease in fH. Reduced rates of gill irrigation generated bursts of respiration-related activity in the cX that were concurrent with activity recorded from the Vth cranial nerve (cn), with the VIIth, IXth and Xth firing later and together. This respiration-related activity appeared to entrain the heart to ventilation. Peripheral burst stimulation of the cX entrained the heart over a range of frequencies, both below and sometimes above the intrinsic fH. These effects indicate that respiration-related changes in fH in pacu can be under beat-to-beat vagal control. Central burst stimulation of respiratory cn VIIth, IXth and Xth also entrained the heart. These data imply that in the normal fish cardiorespiratory interactions are reflexly generated. The central stimulation of the Vth cn was without effect on the heart. The motor neurons of this nerve are the only respiratory motor group not overlapping with cX motor neurons in the dorsal motor column, so that concurrence of bursts of activity in the Vth cn and the cX may arise within the CNS by irradiation from a central respiratory pattern generator.

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#### A5.43

##### Poster Session – Monday 29th June 2009

##### Changing bodies, changing relationships? Heart rate as an indicator of the rate of oxygen consumption throughout the annual cycle of barnacle geese, *Branta leucopsis*

Steven J. Portugal (University of Birmingham), Jon A. Green (University of Liverpool), Phill Cassey (University of Birmingham),

Peter B. Frappell (University of Tasmania), Patrick J. Butler (University of Birmingham)

Quantifying a relationship between heart rate (fH) and rate of oxygen consumption (VO<sub>2</sub>) allows the estimation of VO<sub>2</sub> from fH recorded in free-ranging animals. It has been proposed that this relationship may vary throughout an animal's annual cycle, due to changes in physiology. Barnacle geese, *Branta leucopsis*, provide an ideal model to study this hypothesis, as they exhibit significant intra-annual variability in body mass, body composition and abdominal body temperature, even when in captivity. Fourteen captive barnacle geese were implanted with heart rate data loggers and at six points in the year, the relationship between fH and VO<sub>2</sub> was determined using open-flow respirometry. The fH/VO<sub>2</sub> relationship was also determined in seven wild moulting barnacle geese. In captive barnacle geese, two sessions were significantly different from one another. The relationship between fH and VO<sub>2</sub> obtained from wild geese was significantly different from all of the relationships derived from the captive geese, suggesting that it is not possible to apply calibrations from captive birds to wild geese. However, the similarity of the fH/VO<sub>2</sub> relationship derived during moult in the captive geese to those during the rest of the annual cycle means it is not unreasonable to assume that the relationship between fH/VO<sub>2</sub> during moult in the wild geese is indicative of the relationship throughout the annual cycle.

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#### A5.44

##### Poster Session – Monday 29th June 2009

##### The effect of water-soluble fraction of crude oil on liver of juvenile *Rutilus frisii kutum*

Fatemeh Heidary Jamebozorgi (Post-graduate Student Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University), Behroz Abtahi (Tarbiat Modares University), Eisa Sharifpour (Iranian Fisheries Research Organization), Jafar Seyfabadi (Tarbiat Modares University), Zahra Taghizadeh (Tarbiat Modares University)

The water-soluble fraction (WSF) of crude oil is a small fraction of oil containing components fully soluble in water. Histopathological effect of WSF of crude oil, in different concentrations, on vital organ, liver, of juvenile *Rutilus frisii kutum*, (Cyprinidae), an endemic fish of the Caspian Sea, was investigated. Juvenile specimens weighting approximately 1 g were acclimated to laboratory condition for a minimum of 8 days in a 20-L glass aquarium with dechlorinated water. Prior to the definitive experiment, the phase of the total dissolved WSF in water during 96 h was prepared by using two different concentrations (0.1 and 0.5 of LC<sub>50</sub>) of crude oil WSF. After 24 and 96 h of exposure, 3 individual for each of the two WSF concentrations and three controls were collected for histopathological studies. Liver sections were prepared accordingly and stained using H&E, and then examined by light microscope. Pathological changes in the liver included, irregular shaped hepatocytes, cellular degeneration, Sinusoids expansion and congestion, cytoplasmic vacuolation and congestion of blood vessels. Liver is the main organ for detoxification and biotransformation processes, and due to its function, affected by contaminants in the water. Exposure to crude oil and derivatives can induce a variety of toxic symptoms in experimental animals. This study demonstrated that the WSF of crude oil causes serious damage in the liver of juvenile *R. frisii kutum*.

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**A5.45****Poster Session – Monday 29th June 2009****Anti-oxidant enzyme activity and DNA damage in cultured South African abalone (*Haliotis midae*)**

Anèl Laas (University of KwaZulu-Natal), Daléne Vosloo (University of KwaZulu-Natal), André Vosloo (University of KwaZulu-Natal)

Land based abalone farming in South Africa contributes to the socio-economic well-being of many coastal towns in the Eastern and Western Cape. The environmental oxygen levels on abalone farms are not regulated, causing possible oxidative damage in cultured animals due to inadequate or excessive dissolved oxygen supply.

The aim of this study was to determine to what extent juvenile (average length of  $41.98 \pm 0.61$  mm) and adult (average length of  $65.32 \pm 0.15$  mm) abalone can tolerate long-term (one month) exposure to low or high oxygen levels. Activity of anti-oxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), and DNA damage (using the COMET assay) were investigated after exposure to three oxygen levels.

The GPx activity of the juveniles increased with increasing oxygen levels ( $P < 0.01$ ,  $R^2 = 0.230$ ), where the SOD activity of the adults increased with an increase in oxygen levels ( $P < 0.05$ ,  $R^2 = 0.248$ ). DNA damage (percentage tail DNA and Olive Tail Moment) in the haemocytes of adults exposed to high and low oxygen levels was significantly higher ( $P < 0.01$  and  $P < 0.001$  respectively) compared to adults at intermediate oxygen levels.

Juvenile abalone managed to adapt to changes in their environment, where adult abalone were not able to effectively activate mechanisms to prevent DNA damage.

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**A5.47****Poster Session – Monday 29th June 2009****Behavioural alarm and avoidance responses to copper in rainbow trout (*Oncorhynchus mykiss*): The effect of calcium**

Søren Poulsen (Zoophysiology Dept. of Biological Sciences, Aarhus University), Kim Aarestrup (National Institute of Aquatic Resources, Technical University of Denmark), Jon Christian Svendsen (National Institute of Aquatic Resources, Technical University of Denmark), Hans Malte (Zoophysiology Dept. of Biological Sciences, Aarhus University)

The effect of calcium on the behavioural alarm and avoidance responses of rainbow trout (*Oncorhynchus mykiss*) exposed to copper was tested in a choice tank. When acclimated and exposed in water at low Ca rainbow trout showed increasing avoidance of copper up to a concentration  $20 \mu\text{g Cu/L}$ . At higher concentrations the avoidance response levelled off again and eventually disappeared. Fish acclimated and tested in water at high Ca, on the other hand, avoided all concentrations  $\geq 50 \mu\text{g Cu/L}$ . At avoided Cu concentrations, the average swimming speed distribution in the Cu side shifted towards higher speeds, while that of the clean side shifted towards lower speeds. At low Ca concentrations and high Cu concentrations a decrease in activity was observed even though copper was not avoided. When not given a choice of clean water fish exposed to Cu at low Ca, decreased average swimming speed compared to the control, whereas fish exposed at high Ca, did not change average swimming speed compared to the control. The experiments suggests that the sensory mechanisms responsible for behavioural alarm and avoidance responses are

protected by Ca. The observations that fish being given a choice increased average swimming speed in the Cu side, and that fish not being given a choice decreased average swimming speed, indicate that in rainbow trout at least one alarm response to Cu is context-dependent.

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**A5.48****Poster Session – Monday 29th June 2009****Are there active hearing processes in locust ears?**

Hannah M. Moir (University of Strathclyde), James Windmill (University of Strathclyde)

The locust tympanal organ is a sophisticated example of insect hearing. The tympanal membrane has previously been shown to analyse the frequency of incident sound, such that sound energy is funnelled into waves of motion across the membrane, travelling to frequency specific neurone groups. In vertebrates Otoacoustic emissions have been shown to indicate active hearing processes. These sound emissions are produced as a by-product in the ears of vertebrates, and can be spontaneous or evoked by acoustic stimulus. The evoked emissions, known as distortion-products, are found in active auditory systems when the organ is stimulated with two sounds of different frequencies,  $f_1$  and  $f_2$ . In some insects, including locusts, previous studies have found evidence of distortion-products, but the experimental approach raises questions as to whether the results are actually driven by active hearing processes. In these previous experiments a closed-air system was used with the sound stimulus very close to the organ. The experiments have been revised, altering the experimental set-up to an open system, with appropriate sound pressure levels, such as the ear would expect in a natural setting. A laser vibrometer records the deflection of the locust tympanal membrane at specific points where the mechanoreceptor neurones attach. This allows the membrane to be measured directly for the presence of distortion-products, at the position of the neurones which are believed to be actively generating membrane sound emissions. The measurement, or lack thereof, of distortion-products in the motion of the tympanal membrane is discussed and compared with previous studies.

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**A5.49****Poster Session – Monday 29th June 2009****Using *Drosophila* as a model for the Dipteran Malpighian tubule**

Gayle Overend (University of Glasgow), Debra C. Woods (Pfizer Veterinary Medicine Discovery Research), Shireen A. Davies (University of Glasgow), Julian A. Dow (University of Glasgow),

Despite the bewildering diversity of insect life, renal function appears similar in all species studied. However, how authoritative is this view, based on perhaps half-a-dozen physiological parameters? We compared the transcriptomes of the adult *Drosophila melanogaster* Malpighian tubule and the larval and adult tubules of the malarial vector *Anopheles gambiae* to identify homologous gene pairs which are highly expressed in the tubules of both species. Four gene pairs were chosen for investigation, and Quantitative-PCR, *in situ* hybridization, Western blotting and immunocytochemistry used to

compare expression of each pair of *Anopheles* and *Drosophila* homologues. Further studies utilized the transgenic tools available in *Drosophila* to determine the genes essential for fly development through to adulthood.

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#### A5.50

Poster Session – Monday 29th June 2009

##### Importance of temperature in the molecular and physiological responses to hypoxia in the signal crayfish

Jodie F. Wren (University of Bristol), Steve Morris (University of Bristol), Max Gassmann (University of Zurich), Thomas Gorr (University of Zurich), Dan T. Fagan (University of Bristol),

Aquatic animals are often faced with the challenge of fluctuating O<sub>2</sub> concentration in their environment which can frequently turn hypoxic, and as a result have a suite of defences to cope with O<sub>2</sub> stress. Crayfish exhibit Hypoxia Induced Behavioural Hypothermia (HIBH), whereby hypoxia induces them to seek out an environment with a lower temperature, thereby reducing metabolic rate and the requirement for O<sub>2</sub>. Crayfish exhibit a critical point PO<sub>2</sub> (Pc) below which anaerobiosis is recruited and lactate accumulates. Signal crayfish (*Pacifastacus leniusculus*) were exposed to environmental oxygen levels from normoxia down to 1% O<sub>2</sub> to determine the Pc for physiological responses at 5°, 13° and 20 °C. Respiration rate and key metabolites were measured in haemolymph and muscle tissue (glucose, l-lactate, ATP, ADP, AMP, tissue arginine and arginine phosphate).

Ongoing sequencing work will enhance the EST database fivefold, and from the use of suppressive subtractive hybridisation (SSH) cDNA libraries, sequences will be enriched for those genes over or under-expressed in crayfish exposed to PO<sub>2</sub> below that of the Pc.

This work on the behavioural, physiological and genomic response to hypoxia will be further complemented by proteomic analysis using 2-D DIGE, identification of the differentially expressed proteins by mass fingerprints (MALDI-MS), assessment of Hypoxia Inducible Factor (HIF) and HIBH through HIF hydroxylase inhibition and supplementary experiments to characterise the effects of hypoxia on a crayfish neuronal cell line. NERC (NE/D010845/1).

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#### A5.51

Poster Session – Monday 29th June 2009

##### Using FlyAtlas to detect novel functions for well-known genes in *Drosophila melanogaster*

Stephanie M. Graham (The University of Glasgow), Shireen-Anne Davies (The University of Glasgow), Julian A.T. Dow (The University of Glasgow)

FlyAtlas is an online resource, which allows scientists to look at tissue specific gene expression in the fruit fly *Drosophila melanogaster*. Unexpected expression patterns of previously characterised genes may hint at novel functions, thus helping to close the phenotype gap. To test this hypothesis we looked at the neuronal gene Fasciclin 2 (*fas2*), which has been exhaustively characterised

(over 500 papers), with neural functions ranging from axonal growth in development to synapse stabilization in the adult. Surprisingly FlyAtlas showed *fas2* is predominately expressed in the Malpighian tubule (a renal, rather than neural, tissue), hinting at a previously unreported function in this tissue. Quantitative-PCR validated the extremely high level of transcription reported by FlyAtlas and western blot analysis showed protein expression. Immunocytochemistry and transgenic flies (carrying a GFP or YFP *fas2* construct) show that *fas2* localises to the apical membrane of the tubules, hinting at a role in epithelial polarity.

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#### A5.52

Poster Session – Monday 29th June 2009

##### The importance of respiratory pigments in mediating response to hypoxia in the signal crayfish *Pacifastacus leniusculus*

Elizabeth A. Morgan (University of Bristol), Steve Morris (University of Bristol)

Ectothermic animals (e.g. signal crayfish *P. leniusculus*) may respond to environmental hypoxia by moving to cooler environments, exhibiting “hypoxia induced behavioural hypothermia” (HIBH). One benefit of hypothermia is the concomitant lowering of O<sub>2</sub> demand, preserving internal PO<sub>2</sub> and delaying internal hypoxia. However, thermal acclimation may remove any such benefits. Quantifying the functioning of the respiratory pigment haemocyanin (Hc) is fundamental to understanding its importance in transducing environmental hypoxia and in mediating cellular hypoxia. The response of Hc O<sub>2</sub>-affinity to variations in l-lactate, urate, calcium and magnesium concentrations in the haemolymph were quantified as probable regulators of O<sub>2</sub> binding. Effects of modulators *in vitro* were modelled to aid in the interpretation of Hc function *in vivo*; l-lactate had no effect on O<sub>2</sub>-binding by Hc, but urate, Ca and Mg all potentiated the O<sub>2</sub>-affinity although this was temperature dependent for Ca. The role of temperature in influencing the Hc O<sub>2</sub>-affinity was examined for Hc from crayfish acclimated to 5 °C, 13 °C or 20 °C. Increasing the temperature decreased the O<sub>2</sub>-affinity of Hc, regardless of acclimation history of the crayfish. The *in vivo* functioning of Hc has been assessed by determining the arterial and venous content and partial pressures of O<sub>2</sub> and CO<sub>2</sub>, along with pH. Electrophoresis (SDS-PAGE) is being utilized to assess the effect of temperature acclimation on Hc sub-unit composition. NERC (NE/D010845/1)

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#### A5.53

Poster Session – Monday 29th June 2009

##### Environmental hypoxia: Traits under selection in coastal fish populations

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Faced with environmental change, fish populations respond in one of three ways: 1) they disappear or evade, 2) they remain in their environment and adjust to the new conditions (physiological adaptation) or 3) they adapt (evolutionary adaptation). Nowadays, the differential ability of populations to adapt to environmental change remains poorly understood. At a local scale, fish species currently living in estuaries at the juvenile stage can be submitted to particularly severe hypoxia stemming from anthropogenic activities. Using European sea bass (*Dicentrarchus labrax*), common sole (*Solea solea*) and turbot (*Psetta maxima*), the present experiment aimed at deciphering aspects of local adaptation from phenotypic plasticity. The first phase on the experiment consisted in submitting experimental fish populations to a non-lethal, but acute, hypoxia challenge test and ranking individuals according to their tolerance ability (time at which they displayed respiratory distress). In a second phase we examined the physiology and morpho-anatomy of these fish in order to highlight the potential determinants of the inter-individual difference in hypoxia tolerance.

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#### A5.54

##### Poster Session – Monday 29th June 2009

#### Alarm substance alters early embryonic development in two *Danio* species

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That the abiotic environment can alter aspects of the embryonic development of fish has been recognised for many years. However, less attention has been paid to the effects of fluctuations in biotic factors on early development. Alarm substance, an exogenous chemical cue released into the water upon skin injury in certain fish taxa, can induce premature hatching, changes in adult morphology and behavioural responses in some fish species. The effects of this chemical cue on the plasticity of early fish embryonic development are, however, unexplored. Our study investigated the effects of alarm substance, from skin extracts of adult individuals, on the timings of embryonic development in two species from the family Danioninae (*Danio rerio* and *D. albolineatus*). Embryos were filmed individually for a period of 24 h using the two-cell stage of development as an initial time point. The time of onset of developmental events (i.e. morphological – the eye primordium stage; and physiological – the first muscular contraction and heartbeat) were recorded for both control eggs and those reared in water conditioned with alarm substance. Significant differences in developmental rate resulted from exposure to alarm substance. In particular, *Danio rerio* embryos reared in alarm substance reached the first muscular contraction stage and the initiation of the heartbeat faster than embryos in the control treatment. No significant differences were observed in the order of developmental sequences (heterokairy) within or between species. Cue-related alterations in development may allow embryos to respond to a change in predation pressure.

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#### A5.55

##### Poster Session – Monday 29th June 2009

#### Welfare Implications of stocking density on two fish species (*Paracheirodon innesi* and *Tanichthys albonubes*) kept in home aquaria

Amelia L. Saxby (University of Plymouth), Donna Snellgrove (Waltham Centre for Pet Nutrition), Rod W. Wilson (University of Exeter), Katherine A. Sloman (University of Plymouth)

The aim of this study was to investigate whether stocking density can affect the behaviour and welfare of two fish species regularly kept in home aquaria, neon tetras (*Paracheirodon innesi*) and white cloud mountain minnows (*Tanichthys albonubes*). The capacity of fish to process pain and suffering is still being debated and research into the welfare of fish kept in home aquaria is currently lacking, with no scientifically based guidelines available to hobbyists. This study looked at the darting, aggression and shoaling tendency of fish kept in groups of one, two, five and ten in a 10 L tank and additionally their responses to a novel object. Darting and aggression were lowest in the highest stocking density of both species and the addition of the novel object only affected the darting behaviour of neon tetras. The tendency to shoal was greatest at the highest stocking densities and a correlation between aggression levels and time spent within an area of environmental enrichment was found. We conclude that the optimal welfare of these fish occurs when they are held in groups of at least ten fish per 10 L tank and this density is recommended to hobbyists keeping these species at home. This study forms part of a larger project aiming to produce optimal welfare guidelines for a variety of fish commonly held in mixed-species home aquaria.

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#### A5.56

##### Poster Session – Monday 29th June 2009

#### Modulation of epithelial innate immunity by cyclic nucleotide signalling: Activity of a NFκB orthologue, Relish, is mediated by a cGMP-dependent protein kinase switch

Shireen A. Davies (University of Glasgow), Lorraine Aitchison (University of Glasgow), Gayle Overend (University of Glasgow), Sujith Sebastian (University of Glasgow), Jonathan P. Day (University of Glasgow), Julian A.T. Dow (University of Glasgow)

Epithelial innate immune function is critical for healthy organisms. We investigated cyclic nucleotide signalling in epithelial immunity via IMD pathway activation in the intact *Drosophila* Malpighian tubule, a key nitric oxide (NO)/cyclic(c) GMP-regulated epithelial immune tissue. Anti-microbial peptide (AMP) gene expression and Relish (NFκB orthologue) nuclear translocation assays demonstrate dose-dependent cGMP activation and differential modulation of IMD activity by cGMP-dependent kinases DG1 and DG2, in tubules, but not fat body. Tubule-targeted DG1, activated by immune challenge and NO, enhances Relish translocation/AMP expression, whereas tubule-targeted dg2 transcripts repress Relish translocation/AMP expression, showing close correlation with survival of immune-challenged flies. Potential modulation of the phosphorylation status of Relish by the DG1/DG2 Relish 'switch' was also investigated. This DG1/DG2 'switch' directly affects bacterial gut clearance in bacterial-fed flies,

demonstrating the importance of epithelial cGKs in organismal survival against natural infection.

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#### A5.57

##### Poster Session – Monday 29th June 2009

##### The importance of Crustacean Hyperglycaemic Hormone revealed in novel field determinations on a migratory land crab on Christmas Island

Ute Postel (University of Bristol), Mrinalini Mrinalini (University of Wales), Jessica Palmer (University of Bristol), Lucy Turner (University of Bristol), Simon Webster (University of Wales), Steve Morris (University of Bristol)

Crustacean Hyperglycaemic Hormone (CHH) is implicated in regulating diverse physiological processes, including the mobilization of glucose. The annual breeding migration of the terrestrial Christmas Island red crab, *Gecarcoidea natalis*, requires a fundamental switch in metabolic fuel usage to support several days of walking. The importance of CHH in this change from hypo- to hyperactive states was determined from the circulating CHH titre, in response to physiological and behavioural demands. These measurements required the raising of anti-CHH antibodies and the development of a sufficiently sensitive immunoassay. Tissue metabolite and haemolymph CHH concentrations were measured from crabs sampled during different seasons at different times during the day. An increase in CHH occurred immediately prior to dawn in migrating (wet season) crabs but not in dry season crabs. Complementary laboratory studies showed a pre-dawn hyperglycaemia consequent on this pre-dawn CHH peak. Exercise induced an increase in haemolymph CHH titre which was inhibited by a negative feed-back from elevated circulating glucose. Data from migrating *G. natalis* revealed comprehensive changes in fuel utilization and provided evidence to support a role for CHH in regulating glucose metabolism during exercise under field conditions. The sequences for CHH from *G. natalis* were determined using 3' and 5' RACE. The divergence of the CHH sequences of *G. natalis* compared with those from other species, as compared to the conserved sequence of the related Moulting Inhibiting Hormone implies different selective pressures have shaped the evolution of these peptides, in part perhaps in the adaptation to life on land. NERC (NE/D00246X/1).

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#### A5.58

##### Poster Session – Monday 29th June 2009

##### Retinoic acid signaling in the visual system of the frog *Rana pipiens*: Changes after optic nerve injury

Rosa E. Blanco (Institute of Neurobiology), Wilmer Rivera (Institute of Neurobiology), Alma M. Mas (School of Medicine), Jonathan M. Blagburn (Institute of Neurobiology), Mildred Duprey-Diaz (Institute of Neurobiology)

Retinoic acid (RA) is a potent transcriptional activator that may be involved in neuronal plasticity and regeneration in the adult brain.

RA is synthesized by the enzyme retinaldehyde dehydrogenase (RALDH), its availability is regulated by the cellular retinoic acid binding proteins (CRABP-I and CRABP-II), and its various effects are mediated via the retinoic acid receptors (RARs). The purpose of this study is to understand the role of RA during regeneration in the adult visual system of *Rana pipiens*. Immunocytochemistry was performed on cryostat sections of the retina and optic tectum of control and experimental animals whose optic nerves were severed, using antibodies against RALDH, RAR $\alpha$ , RAR $\beta$ , CRABP-I and II. In control retinas, immunoreactivity to RALDH, both RARs, and both CRABPs was present in cells of the inner nuclear layer and ganglion cell layer. CRABP-I and II were also observed in cells of the outer nuclear layer, while the inner and outer plexiform layers, and some Müller-like processes, showed staining for CRABP-I and RALDH. A transient increase in the immunostaining of RALDH and RARs was observed in retinas during regeneration. Moderate to low staining for RALDH, RAR $\alpha$  and RAR $\beta$  was present in control tectum, and an increase was observed during regeneration and reconnection. A few neurons appeared to have moderate staining for CRABP-I, while high levels of CRABP-II were maintained in tectal cells during regeneration. These results are consistent with the idea that adult visual system neurons may reuse developmental signaling mechanisms in response to axonal injury.

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#### A5.59

##### Poster Session – Monday 29th June 2009

##### Functional role of $\beta_3$ -adrenergic receptors on in vivo cardiac function in freshwater teleosts

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Historically, the  $\beta_2$ -adrenergic receptor has been widely studied due to its role in teleost cardiac function. Recently, a novel subtype,  $\beta_3$ -adrenergic receptor, has been identified in the teleost heart, however there is no information regarding the in vivo functional role of this novel receptor subtype in teleosts. Studies with channel catfish (*Ictalurus punctatus*) showed that injection of the  $\beta_3$ -AR specific agonist BRL37344 (3 mg/kg) induced a ~50–60% increase in cardiac output (Q) and stroke volume (Sv), as well as a 20% increase in heart rate ( $f_H$ ). However, injection of the  $\beta_3$ -AR specific antagonist (SR59230) resulted in a decrease in cardiac inotropy. We observed a 10% decrease in Sv which was accompanied by a ~20% increase in  $f_H$  resulting in a slight increase in Q. Interestingly, the functional role of  $\beta_3$ -AR appears to be developed in the early life stages of fish, as we found a significant increase in both Sv and Q and a slight increase in  $f_H$  following 30 min exposure of fathead minnow (*Pimephales promelas*) larvae to BRL37344 (100  $\mu$ M). On the contrary, SR59230 exposure (10  $\mu$ M) resulted in a significant decrease in  $f_H$ , which was accompanied by a significant increase in Sv and an increased Q. Further studies on Japanese medaka (*Oryzias latipes*) larvae and adult rainbow trout are currently underway. Our study shows that our current knowledge regarding the behaviour and functional role of  $\beta_3$ -AR in the lower vertebrate heart warrants further investigation.

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**A5.60****Poster Session – Monday 29th June 2009****Physiological function of R–L shunt in the American alligator**

Ashley S. Vorhees (University of California Irvine), John Eme (University of California Irvine), June C. Gwalthery (University of California Irvine), Tomasz Owerkowicz (University of California Irvine), James W. Hicks (University of California Irvine)

All non-avian reptiles possess heart morphologies that allow for mixing of oxygenated and deoxygenated blood (cardiac shunts). The physiological significance of pulmonary to systemic (R–L) shunting in crocodylians was assessed in American alligators (*Alligator mississippiensis*) by surgical occlusion of the shunt, followed by measurement of physiological parameters. To eliminate the capacity for R–L shunt, the left aortic arch (LAo) was surgically occluded in 36 alligator hatchlings. The cardiovascular effects of shunt removal were examined by measuring cardiac pressures and flows as well as changes in ventricular morphology. Physiological performance was assessed by measuring growth (body mass, total length, snout-vent length) over the 18-month period following surgery, as well as voluntary dive times and exercise capacity (treadmill and swimming). Measurements show that removal of R–L shunt in crocodylians significantly increases peak systolic pressures in both the right and left ventricle and induces marked enlargement of the right ventricle (mean increase 70%). However, removal of R–L shunt showed no significant effect on growth rates, voluntary dive time or exercise capacity. Based on these data, we suggest that while removal of the LAo altered ventricular hemodynamics (evidenced by increased ventricular pressures and ventricular enlargement), removal of the R–L shunt does not appear to have any significant effects on overall animal performance. Supported by NSF Grant # IOB0445680 to JWH.

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**A5.61****Poster Session – Monday 29th June 2009****Tracing the origin of different red blood cell NKCC activation modes within birds**

Pia Koldkjær (University of Liverpool), Augusto S. Abe (UNESP Rio Claro), Tobias Wang (University of Aarhus), Mads F. Bertelsen (University of Copenhagen), Arjen Van't Hof (University of Liverpool)

The red blood cell (RBC)  $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$  cotransporter (NKCC) is involved in cell volume regulation and may buffer  $\text{K}^+$  released from exercising muscles, thereby aiding the maintenance of cardiac excitability and delaying fatigue. It is activated by cell shrinkage and low blood oxygen levels in different vertebrates, including mammals. Additionally, in birds, catecholamines initiate a signal transduction cascade stimulating the NKCC through final phosphorylation by cAMP-PK. We use a comparative approach to attempt to elucidate the physiological role(s) of NKCC in bird RBCs. NKCC activity was measured as bumetanide-sensitive unidirectional  $^{22}\text{Na}$  influx in response to osmotic RBC shrinkage, deoxygenation by  $\text{N}_2$  incubation, and stimulation by the  $\beta$ -adrenergic agonist isoproterenol ( $10^{-5}$  M) at physiological pH and temperature. We show that several bird species lack  $\beta$ -adrenergic NKCC stimulation. Shrinkage may be the most ancient activation mode within birds, being the only stimulus that activated NKCC in ostrich, belonging to the most basal extant bird lineage. We have sequenced and identified regulatory phosphorylation sites in bird NKCCs with different activation modes. We found identical regulatory domains, suggesting that differences in signal

transduction are responsible for observed activation patterns. Lack of NKCC activation by any of the common physiological stimuli in the reptilian out-group indicates independent evolution of NKCC activation in birds and mammals.

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**A5.62****Poster Session – Monday 29th June 2009****The effect of PCBs on the startle response in common sole juveniles (*Solea solea* L.)**

Fabrizio Atzori (FREDD, CNRS-University of La Rochelle-Ifremer, France), Marcella Cannas (LIENSs, UMR 6250 CNRS, University of La Rochelle, France), Paolo Domenici (CNR-AMC, Italy), Christel Lefrancois (LIENSs, UMR 6250 CNRS, University of La Rochelle, France)

Like many juvenile teleosts, common sole utilize coastal zones as nursery grounds. However, in some coastal areas, increasing anthropic pressure induces a decrease in water quality due to a number of factors including chemical contamination (e.g. PCBs). Sole juveniles are particularly vulnerable to coastal pollution, because of their lifestyle and their limited swimming ability at this stage. Our aim was to investigate the effect of PCBs on the response to a startle stimulus in sole <1 year old. Our hypothesis was that PCBs may affect the sole's response by impairing their ability to detect startle stimuli and/or their locomotor performance. An integrative approach was therefore employed by measuring locomotor variables (i.e. maximum swimming speed and acceleration, turning rate and escape trajectory), variables related to sensory performance (i.e. responsiveness), as well as physiological variables (i.e. ventilation frequency). The experiments were carried out on individuals fed during 30 and 60 days with contaminated food. Sole were exposed to four treatments: sole fed with uncontaminated food, sole fed with pellets mixed with solvent (utilized to fix the PCBs) and two groups fed with pellets contaminated with different concentrations of PCBs ( $\Sigma\text{PCB} = 600 \mu\text{g PCB/kg}$  of food;  $2100 \mu\text{g PCB/kg}$  of food). To induce the startle response, a dummy simulating an aerial predator was dropped into the water. Results will be discussed considering the different types of responses showed by the sole (i.e. no response, freezing or escape response), as well as the performance showed during escape responses.

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**A5.63****Poster Session – Monday 29th June 2009****Effect of induced mild hypothermia upon the dynamics of sepsis progression in rat CLP model**

Karelle Leon (Laboratoire ORPHY, Université Européenne de Bretagne, Université de Brest), Eric Quemener (Laboratoire ORPHY, Université Européenne de Bretagne, Université de Brest)

Septic shock is the main cause of mortality in intensive care units. Sepsis is a systemic inflammatory response to infection which leads to tissue hypoperfusion, hypotension and multiple organ dysfunction. Pathological and physiological changes such as cardiac output impairments, core temperature abnormalities, exaggerated

inflammatory response, disseminated intravascular coagulation and lactic acidosis have been shown to correlate with mortality in patients with septic shock.

Previous studies in our laboratory have shown that induced mild hypothermia significantly increased the survival of septic rat. Trying to explain how hypothermia could slow down the progression of sepsis, we measured arterial blood gas (PaO<sub>2</sub>, PaCO<sub>2</sub>), pH, [HCO<sub>3</sub><sup>-</sup>], haemoglobin content as well as plasma lactates and cytokines (IL6 and TNF alpha) as indices of oxygenation status, ventilatory efficiency, acid–base balance, energy metabolism and inflammatory state.

The study was conducted using 84 male Sprague–Dawley rats which were anaesthetized and maintained under normothermic (38 °C) or mild hypothermic (34 °C) conditions. Rats were assigned to one of four groups (septic + hypothermia, sham-control + hypothermia, septic + normothermia and sham-control + normothermia). Sepsis was experimentally induced by a cecal ligation and puncture (CLP). The sham-control groups underwent the same procedures but the cecum was neither ligated nor incised. At T0 and after 2, 4 or 6 h, rats were sacrificed and the arterial blood immediately withdrawn for assays.

It is expected that our results, correlating the induced hypothermia with blood chemistry and oxygenation conditions, will reinforce the take care of sepsis patients in intensive care units.

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#### A5.64

Poster Session – Monday 29th June 2009

#### Autonomic regulation of the heart during digestion and swimming in the European sea bass (*Dicentrarchus labrax*)

Nina K. Iversen (Aarhus University), Aurélie Prinnet (Université Montpellier 2), Inge Findorf (Aarhus University), David McKenzie (Université Montpellier 2), Tobias Wang (Aarhus University)

The cardiovascular responses to digestion and exercise and their autonomic regulation were studied in the European sea bass (*Dicentrarchus labrax*). We measured cardiac output (*Q*), heart rate (*f<sub>H</sub>*) and oxygen consumption (MO<sub>2</sub>) at rest and during digestion or exercise and inhibited the autonomic innervation of the heart by intraperitoneal infusion of antagonists (β-adrenergic, propranolol and muscarinic cholinergic, atropine, 5 mg kg<sup>-1</sup> each). Digestion was associated with a pronounced tachycardia, a decrease in stroke volume (*V<sub>s</sub>*), resulting in a slight elevation of *Q* (61.9 ± 5.5 to 68.0 ± 5.4 ml min<sup>-1</sup> kg<sup>-1</sup>) compared to rest. Swimming caused a marked increase in *Q* (42.7 ± 6.3 to 60.9 ± 3.6 ml min<sup>-1</sup> kg<sup>-1</sup>) with increasing swimming speed (20 to 60 cm s<sup>-1</sup>), through a rise in *f<sub>H</sub>* while *V<sub>s</sub>* did not change.

Resting heart rate was characterized by a pronounced inhibitory vagal tone (98.3 ± 21.7%), and the elevation in heart rate when metabolism increased was mainly due to a reduction of the vagal tone. Thus, adrenergic tone remained at 30–40% during rest, exercise and digestion. Intrinsic *f<sub>H</sub>*, revealed after double autonomic blockade was similar during rest and digestion (71.1 ± 4.5 and 69.8 ± 6.3 min<sup>-1</sup>). This demonstrates that non-adrenergic, non-cholinergic (NANC) factors are of little importance in mediating the heart rate response to digestion in this species, it may be mechanical distention of the stomach wall which mediates the decreased cholinergic tone.

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#### A5.65

Poster Session – Monday 29th June 2009

#### Bimodal regulation of heart rate during hypoxic bradycardia in rainbow trout *Oncorhynchus mykiss*

Mikkel Boel (Zoophysiology Dept. of Biological Sciences, Aarhus University), Hans Malte (Zoophysiology Dept. of Biological Sciences, Aarhus University)

Rainbow trout were acclimated to normoxia or severe hypoxia (25% O<sub>2</sub> saturation) and subsequently exposed to various degrees of hypoxia ranging from 90%–25% while the ECG and buccal pressure was recorded. The severity of the hypoxic exposure was intensified in a stepwise manner in stages of 40 min duration. The classical responses to hypoxia, i.e. progressive increases in ventilation rate (*f<sub>R</sub>*) and amplitude as well as bradycardia, were observed in both acclimation groups, even though bradycardia, appeared only at the more severe hypoxia levels (40% and 25%). However, histograms of the distribution of the beat to beat intervals (H–H) revealed that, in most individuals of both acclimation groups, regulation of the heart occurred at less severe hypoxia levels. Thus, in normoxic exposures, there was a unimodal H–H distribution which, under moderate hypoxia levels, became narrower and progressively moved to shorter H–H intervals (tachycardia). At a hypoxia level around 60% or 50% a second peak of larger H–H intervals appeared, which gradually grew, while the preceding peak of smaller H–H intervals gradually became smaller. At return to normoxia the original, unimodal distribution was re-established. Thus, it seems that bradycardia results from a shifting balance between groups of H–H intervals.

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#### A5.66

Poster Session – Monday 29th June 2009

#### Postprandial cardiac constancy in ball pythons, *Python regius*

Bjarke Jensen (Aarhus University)

Pythons are renowned for their pronounced metabolic response to digestion and a marked growth of the visceral organs during the postprandial period. The heart of Burmese pythons (*Python molurus*) may grow by up to 40% 48 h after ingestion of large meals, but very little is known about possible changes in the gross morphology. To investigate whether the morphology of the python heart changes during digestion (fed 25% of their body mass), we compared heart structure of ball pythons (*Python regius*) during fasting and at 24 and 48 h into the postprandial period (*N* = 5 in each group). Dry mass of the small intestine (SI), kidneys (K) and liver (L) increased significantly during digestion (48 h to fasting relative organ dry-weight increase was; SI = 157.2%; K = 47.3%; L = 47.5%; *N* = 5 per group). However, there was no significant changes in cardiac ventricular dimensions (length, width and volume; *N* = 5 per group) or wet mass relative to body mass (fasting, 0.27%; 24 h, 0.27%; 48 h, 0.28%; *N* = 3 per group). The hearts were sectioned in 2 mm transverse slices (10–12 slices per ventricle) to inspect the three major ventricular septa, the muscular ridge, the bulbuslamelle and the vertical septum that are responsible for the unique pressure separation of pythons. None of the septa showed distinct changes. It appears that the postprandial cardiac growth is less pronounced in ball pythons compared to Burmese pythons and we speculate that the

different species of pythons have several mechanisms to fulfil the increased demands on oxygen delivery during the postprandial period.

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#### A5.67

##### Poster Session — Monday 29th June 2009

##### ABC transporters in the *Daphnia pulex* genome: Implications for ecotoxicology and drug resistance in crustacean parasites

Armin Sturm (Institute of Aquaculture, University of Stirling), Stephen S. George (Institute of Aquaculture, University of Stirling), Michael Dean (National Cancer Institute, Frederick, MD, USA), Phil Cunningham (King's College London), Anke Treuner-Freeman (Institute of Aquaculture, University of Stirling)

The large gene superfamily of ABC (ATP-binding cassette) transporters encodes membrane proteins involved in trafficking processes across biological membranes and further cell biological functions. ABC transporters are evolutionary ancient and involved in the biochemical defence against toxicants. The increased expression of ABC transporters has been suggested as an adaptive biochemical mechanism enabling animals to survive in polluted environments, or conferring resistance against drugs in parasites.

Here, we report the first genome-wide survey of ABC proteins in a crustacean, obtained by screening of the recently sequenced *Daphnia pulex* genome. 64 ABC proteins were found in the *Daphnia* genome, covering all current ABC subfamilies A to H. Phylogenetic analyses with ABC proteins of yeast, worm, fruit fly and human revealed a comparatively high degree of evolutionary conservation in ABC transporters involved in fundamental cellular processes, including the mitochondrial half transporters of the ABCB subfamily, and the members of subfamilies ABCD, ABCE and ABCF. In the remaining ABC subfamilies, lineage specific gene duplications and gene losses were frequent. *Daphnia* showed two proteins homologous to MDR (multidrug resistance) P-glycoproteins (ABCB subfamily) and six proteins homologous to MRPs (multidrug resistance-associated proteins) (ABCC subfamily). Using *Daphnia* ABC protein sequences to derive PCR primers, we isolated related partial cDNAs in the sea louse (*Lepeophtheirus salmonis*), a fish ectoparasite causing considerable economic losses in salmon farming. Further studies are required to elucidate whether the sea louse ABC transporters play a role in its susceptibility to antiparasitic drugs.

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#### A5.68

##### Poster Session — Monday 29th June 2009

##### Differences in sensitivity to waterborne Cu in three freshwater fish: Disturbance of hormonal processes related to ionoregulation

Marleen Eyckmans (University of Antwerp), Veerle Darras (Catholic University of Leuven), Ronny Blust (University of Antwerp), Gudrun De Boeck (University of Antwerp)

Different sensitivities of fish towards metal pollution are species specific but underlying mechanisms are often not well understood.

Copper induces stress and disturbs ionoregulation, which are both under hormonal control. Therefore we exposed 3 fish species to 2 copper levels and examined cortisol, thyroid and prolactin metabolism. In a first exposure we aimed at equal toxicity levels and exposed fish to 10% of their respective 96 h LC50 value (20 µg/L for rainbow trout, 65 µg/L for common carp and 150 µg/L for gibel carp). In a second series we exposed the fish to an equal copper concentration: the Flemish water quality criterion for surface waters (50 µg/L). After 1 h, 12 h, 24 h, 3 days, 1 week and 1 month, plasma cortisol and thyroid concentrations were measured and in gill tissue, the expression of thyroid and prolactin receptors was investigated.

Copper exposure affected hormone levels and clear differences between fish species were evident. Rainbow trout and common carp thyroid metabolism mainly experienced adverse effects at the beginning, while gibel carp showed a slower response in the middle of the experiment. Common carp also displayed a change after 1 month. Cortisol concentrations revealed hardly any difference between control and exposed fishes, mainly due to relatively high control levels. The prolactin receptor showed alternating up and down regulation of the prolactin receptor in rainbow trout and common carp and a downregulation in gibel carp.

In conclusion, the disturbance of hormonal processes due to metal toxicity, displayed pronounced differences between the studied fish species.

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#### A5.69

##### Poster Session — Monday 29th June 2009

##### Revisiting the icefish cardiocirculatory compensations in the era of nitric oxide

Filippo Garofalo (University of Calabria), Bruno Tota (University of Calabria), Daniela Amelio (University of Calabria), Daniela Pellegrino (University of Calabria)

Very recent genome-wide investigations of transcriptional and genomic changes on Antarctic notothenioid cold adaptation support the idea that this fish group underwent striking genomic expansion of specific protein gene families, enhancing physiological fitness in near freezing waters (Chen et al., 2008). This process was accompanied by dramatic gene loss, as epitomized by the Channichthyidae (icefish), unique example of disadaptation for their loss of haemoglobin (Hb) and, in some species, cardiac myoglobin (Mb), once considered essential-life oxygen-binding chromoproteins. In an integrative frame, we review here aspects of icefish gene expression reprogramming and compensatory responses that have shaped their alternative cardio-circulatory design, Hb-free blood and Mb-free cardiac muscle. We revisit the physiological significance of the multilevel cardio-circulatory compensations that counteract the challenge of hypoxemic hypoxia by increasing peripheral oxygen transcellular movement for aerobic tissues, including the myocardium. The advent of nitric oxide (NO) biology prompts to reconsider both polar cold adaptation and icefish redox homeostasis in a new perspective, NO acting as a major integrator of the multilevel cardio-circulatory adjustments, compensating disadaptation with functional phenotypic plasticity. We also show that, beside NOS-dependent NO generation (Garofalo et al., 2009), the newly uncovered Hb/Mb interplay with NO and nitrite has revealed unexpected hemoprotein functions, suggesting that both Hb and Mb are involved as nitrite reductases in several icefish cardiocirculatory processes (Cerra et al., in press). On the whole, this opens new horizons in evolutionary physiology.

Cerra, et al., in press. BBA.

Chen, et al. PNAS, 105: 12944–12949, 2008.

Garofalo, et al. Nitric Oxide, 20:69–78, 2009.

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#### A5.70

**Poster Session – Monday 29th June 2009**

##### **Role of Angiotensin II in fish cardiac remodelling**

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Angiotensin II (AngII) is a pluripotential hormone whose actions include short-term modulation (in eel, see Imbrogno et al., 2003) and long-term adaptations. While in vertebrates a variety of species-dependent variations is evident in the short-term contractile influence of AngII, there is a lack of information regarding its cardiac remodelling role in fish. This was explored in the present study using isolated heart preparations of freshwater eel (*Anguilla anguilla* L.), in which the mechanical performance was characterized on the basis of the Frank–Starling response. Myocardial changes were analysed in controls and in counterparts intraperitoneally injected with AngII for 15 days. Both groups showed similar Frank–Starling responses. However, while afterload increases in control hearts rapidly decreased stroke volume, this was better maintained in the AngII-treated counterparts. Noteworthy, the AngII-elicited effect was abolished by AT<sub>2</sub> receptor antagonist (CGP<sub>42112</sub>) pre-treatment. These results, together with parallel morphological findings, point to a role of AngII in cardiac remodelling of the eel heart, providing new insights regarding modulation of cardiac plasticity in fish.

Imbrogno, et al. J. Exp. Biol. 206: 2675–2684, 2003

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#### A5.71

**Poster Session – Monday 29th June 2009**

##### **Catestatin is a new modulator of cardiac performance in fish**

Bruno Tota (University of Calabria), Filippo Garofalo (University of Calabria), Sandra Imbrogno (University of Calabria), Sushil K. Mahata (University of California), Bruno Tota (University of Calabria)

The ubiquitous glycoprotein Chromogranin A (CGA), secreted with catecholamines upon chromaffin cell stimulation, through cell-, tissue- and species-specific proteolytic processes generates several regulatory peptides (vasostatins, pancreastatin, catestatin, parastatin) which are powerful inhibitors of endocrine secretion (Helle et al., 2007). Catestatin (Cts), the small 21-aminoacid-long peptide, inhibits the nicotinic-induced catecholamine release, is a potent vasodilator, anti-hypertensive agent in mammals, including humans. We recently discovered that it functions as an important cardio-suppressive modulator of the frog (Mazza et al., 2008) and the rat (Angelone et al., 2008) heart. In an evolutionary perspective, we report now Cts (bovine CGA344–364) influence on isolated

and perfused eel (*Anguilla anguilla* L.) heart preparations to explore its cardiotropic role in fish and the eventual species-specific mechanisms underlying its myocardial action. Stroke volume (SV) and stroke work (SW) were used as measure of inotropism. Concentration–response curves were generated exposing the hearts to increasing doses of Cts; inhibitors of the nitric oxide (NO) signaling were also used to study its involvement in the Cts-induced effects. Under basal (unstimulated) conditions, Cts caused a concentration-dependent negative inotropic effect which, as in frog and rat hearts, was NO-dependent. The Cts influence was also studied under chemically- and physically-stimulated conditions and the patterns compared with those obtained in frog and rat. The data strongly support an early role of Cts as a cardio-suppressive peptide in vertebrates.

Angelone, et al. Endocrinología 49: 4780–4793, 2008.

Helle, et al. Cell. Mol. Life Sci. 64: 2863–2886, 2007.

Mazza, et al. Am. J. Physiol. 295: 113–122, 2008.

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#### A5.72

**Poster Session – Monday 29th June 2009**

##### **Control of the coronary microcirculation in steelhead trout (*Oncorhynchus mykiss*)**

Isabel Costa (Memorial University of Newfoundland), Travis W. Hein (Texas AM Health Science Center), Kurt Gamperl (Memorial University of Newfoundland)

Changes in tissue perfusion/vascular resistance are primarily mediated by the dilation or constriction of vessels ranging from 5 to 150  $\mu\text{m}$  in diameter. Mammalian researchers have used isolated microvessel techniques for nearly two decades to directly examine how various parameters affect the dilation and constriction of these vessels without the complex interactions that exist in intact organs/tissues. In contrast, studies of fish vascular physiology have largely been restricted to larger conductance vessels. Therefore, we have begun a program of research on microvessel physiology, and are currently investigating how trout isolated coronary microvessels (<150  $\mu\text{m}$  i.d.) respond to known vasodilators and constrictors, and whether acclimation temperature (10, 5 and  $1 \pm 1.0$  °C) influences these responses. Our initial experiments show that coronary microvessels respond with a concentration-dependent ( $10^{-10}$ – $10^{-4}$  M) dilation to adenosine, sodium nitroprusside and serotonin; whereas a biphasic response (constriction followed by dilation) is observed in vessels exposed to increasing concentrations of norepinephrine and epinephrine. Interestingly, weak constriction was seen with acetylcholine, and endothelin-1 only constricted coronary microvessels at the highest dose ( $10^{-5}$  M). At present, we have only compared the effect of acclimation to 10 vs. 5 °C on the magnitude of the microvessel response to adenosine and norepinephrine. However, these results indicate that temperature significantly decreases the response of microvessels to these two compounds. We will present results from these experiments, and those from work that is currently ongoing.

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**A5.73****Poster Session – Monday 29th June 2009****Toxicological effects of dispersed crude oil on Golden Mullet *Liza aurata* innate immune function**

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Dispersants transfer oil from the water surface into the water column and consequently modify hydrocarbons petroleum fish uptake. It appears that toxicity will depend on whether oil dispersion increases or decreases the exposure of aquatic species to the toxic components of oil.

Lethal bioassays were conducted on *Liza aurata*. During 48 h, fish were exposed under several pathways of oil contamination: water soluble fraction of oil (WSF), a chemically dispersed crude oil and a mechanically dispersed crude oil. In addition, a control condition with dispersant was evaluated. Hydrocarbons concentrations were determined along the exposure period (on their soluble and particular fraction). Following this period, a 14 days recovery period was conducted.

Because understanding changes in fish immune system is relevant for evaluating the health of the aquatic environment, our study deals with modification of its response by recognised biomarkers such as the haemolytic activity of alternative complement pathway (ACH50). In addition, we studied gill surface and hepatic morphological histology to better understand the innate immunity alteration. Furthermore, physiological parameters including condition factor and liver somatic index, biotransformation enzymes such as 7-ethoxyresorufin-*O*-deethylase (EROD) and glutathione-*S*-transferase, antioxidant enzymes including glutathione peroxidase and total glutathione (GSH) content and lipoperoxidation (as TBARS) were measured in these conditions. These responses were correlated to the exposure level by bioaccumulation measurements before and after the decontamination period.

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**A5.74****Poster Session – Monday 29th June 2009****Effects of injected nanoparticles on blood pressure in mice**

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Nanoparticles have an immense clinical potential because they, by virtue of their small size, can be injected into the blood stream and dispersed to reach specific targets within the body. One such example is the wish to develop coated iron oxide ( $\text{Fe}_3\text{O}_4$ ) nanoparticles that bind to specifically to cancer tumors where they can be activated to produce heat and kill the tumor tissue. As part of this initiative, it is imperative to investigate whether the injected nanoparticles interfere with the endothelium and weaken endothelial regulation of vascular tone and blood pressure. We evaluated the

effect of injected  $\text{Fe}_3\text{O}_4$  (uncoated, spherical 25 nm APS) with a purity of 99.5% on heart rate ( $f_H$ ) and mean arterial blood pressure (MAP). The mice were anaesthetized by an intraperitoneally injection of hypnorm-diazepam ( $0.315$  and  $5 \text{ mg ml}^{-1}$ , respectively) at  $0.01 \text{ mg kg}^{-1}$  and kept anaesthetized throughout the entire experimental period. A catheter was inserted in the right carotid artery for measurements of  $f_H$  and MAP. One hour subsequent to surgery, nanoparticles suspended in 10% saline (0.9%) and 3% mouse serum were then injected in the tail vein in three increasing doses ( $0.33$ ,  $0.63$ ,  $0.95 \text{ mg kg}^{-1}$ ) and the cardiovascular parameters were measured. Overall, injections of nanoparticles at these dosages did not affect MAP or  $f_H$  indicating that iron oxide particles do not appear to interfere with endothelial function.

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**A5.75****Poster Session – Monday 29th June 2009****An immunohistochemical study of gill epithelium cells in the Nile tilapia, *Oreochromis niloticus***

Sandra M. Monteiro (CITAB – Center for the Research and Technology for Agro-nvironmental and Biological Sciences), Sofia Garcia-Santos (CITAB – Center for the Research and Technology for Agro-nvironmental and Biological Sciences), António Fontainhas-Fernandes (CITAB – Center for the Research and Technology for Agro-nvironmental and Biological Sciences), Mário Sousa (ICBAS, University of Porto)

Disturbance of the structure of gill compromises the survival of fish since relevant physiological processes take place in this organ. Clarification of the normal histology of a particular species is of supreme importance in order to interpret the morphological and functional modifications occurring under pathological conditions. The histochemistry or immunohistochemistry are valuable tools in the identification and functional characterization of different cell types. This is the first work reporting a complete mapping of the gill epithelium in Nile tilapia, *Oreochromis niloticus*, using these techniques. The results showed that the stratified filament epithelium of *O. niloticus* gill can be divided in a superficial layer, where pavement, mucous and mitochondria rich cells can be found, and a deep layer, constituted by undifferentiated, myoepithelial-like, granular and neuroendocrine cells. Antibodies against V-ATPase and  $\text{Na}^+/\text{K}^+$ -ATPase allowed the identification of pavement and mitochondria-rich cells, respectively, suggesting that, in *O. niloticus*, pavement cells are implicated in  $\text{Na}^+$  uptake. The use of PAS and Alcian Blue with different pH ranges allowed the recognition of distinct sub-populations of mucous cells. Neuroendocrine markers such as Grmelius, Synaptophysin and Serotonin were detected in different cell types, suggesting a neuroendocrine role for mitochondria-rich cells and distinct neural pathways, a putative  $\text{O}_2$ -chemosensory and an ion regulatory pathway. The presence of resident giemsa positive-granular cells indicates a possible defence role to the deep filament epithelium. Proliferating cell nuclear antibody recognized two different cell types, undifferentiated and myoepithelial-like cells. In the superficial layer, it is here stated for the first time the existence of vimentin positive cells.

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**A5.76****Poster Session – Monday 29th June 2009****Effects of different source and level of protein in feed mixtures on liver and intestine histology of the common carp (*Cyprinus carpio*, Linnaeus, 1758)**

Bozidar Raskovic (University of Belgrade, Faculty of Agriculture), Marko Stankovic (University of Belgrade, Faculty of Agriculture), Zorka Dulic (University of Belgrade, Faculty of Agriculture), Zoran Markovic (University of Belgrade, Faculty of Agriculture), Nada Lakić (University of Belgrade, Faculty of Agriculture), Vesna Poleksic (University of Belgrade, Faculty of Agriculture)

In order to investigate effects of different feed mixtures in extruded feed for carp two 90 days feeding experiments on common carp yearlings (*Cyprinus carpio*, Linnaeus, 1758) were carried out. Effects on digestive tract morphology were studied. The study was performed in the Laboratory for fish nutrition at the Faculty of Agriculture, University of Belgrade. In the first experiment diets with different levels of fishmeal and soybean meal were used. In the second experiment the level of proteins in fishmeal varied. Distal intestine and liver of experimental carp were sampled at the start and after 90 days of the experiment. In both experiments no major pathological changes were recorded, therefore we used some morphometric parameters for the estimation of histological status of two organs which are usually most affected by different diets – liver and the intestines. In liver, average nuclear area was measured and compared between fish fed different diets. In the intestines, quantification of enterocytes height, and length of intestinal folds was performed. The results of histological and morphometrical analysis are discussed in relation with feed composition. This study suggests that histological and morphometric analyses can be useful additional tools when feed efficiency is being investigated in fish.

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**A5.77****Poster Session – Monday 29th June 2009****Development of enrichment criteria for zebrafish (*Danio rerio*) used in laboratory studies**

Luanne Wilkes (University of Exeter), L. Leaver (University of Exeter), Kath Sloman (University of Plymouth), G. Readman (AstraZeneca), T. Williams (AstraZeneca), S. Owen (AstraZeneca), Rod Wilson (University of Exeter)

At present there is little available information concerning fish welfare within laboratory studies, beyond that concerning the environmental conditions required for their survival. As such there is currently no legislation regarding provision of any forms of environmental enrichment such as is commonly recommended for mammals and birds.

To study the possible benefits of enrichment for juvenile zebrafish (*Danio rerio*), we kept two groups of fish in either “enriched” or “barren” tanks. Those classified as enriched contained 3-D glass rod structures which fish could swim between and around in order to provide them both with a more complex environment and a refuge from conspecifics and human observers.

During the study, behavioural data relating to fish activity levels, shoaling density and use of enrichment, was collected and analysed. Results of this data seem to suggest that the enrichment provided has no discernible effect on either activity levels or shoaling density of

fish. Importantly, fish did not spend a significantly greater or lesser amount of time in areas containing enrichment than those without.

Following termination of the study, fish whole bodies are being analysed for both cortisol and ion levels to determine if provision of enrichment has any effect on these commonly used physiological indicators of stress. In addition to this, to account for any possible effects of ontological change on the benefits of need for enrichment, we are now repeating the study using the mature siblings of the original fish tested.

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**A5.78****Poster Session – Monday 29th June 2009****Effects of dispersed crude oil upon the cardiovascular physiology and the metabolic scope of juvenile Golden Mullet *Liza Aurata***

Thomas Milinkovitch (Littoral Environnement Sociétés (LIENSs), UMR 6250 CNRS, University of La Rochelle), C. Le François (Littoral Environnement Sociétés (LIENSs), UMR 6250 CNRS, University of La Rochelle), J. Lucas (Littoral Environnement Sociétés (LIENSs), UMR 6250 CNRS, University of La Rochelle), Hélène Thomas Guyon (Littoral Environnement Sociétés (LIENSs), UMR 6250 CNRS, University of La Rochelle), S. LeFloch (Cedre), N. Imbert (Littoral Environnement Sociétés (LIENSs), UMR 6250 CNRS, University of La Rochelle)

Dispersants used in case of oil spills aim at transferring oil from the water surface into the water column. Consequently, when dispersants are applied before the oil slick reaches the coastline, the hydrocarbons bioavailability increases for aquatic organisms living in water-column or on sea floor. Our study aimed at assessing the effects of the oil dispersion in *Liza aurata*, a species living in coastal areas. During 48 h, fish were exposed to different conditions which could occur during an oil spill: to the dispersants itself, to the water soluble fraction of oil (WSF), to a chemically dispersed crude oil and to a mechanically dispersed crude oil. In addition, a control condition was tested. Hydrocarbons concentrations were analysed all along the exposure period (on both their soluble and particular fraction). After 48 h-exposure to one of these treatments, some key physiological functions were assessed in *L. aurata*. At the heart level, myocardium performances were evaluated, through measurements of the contractility of ventricular muscle strips. The efficiency of the adrenergic pathway and the calcium handling capacity by the sarcoplasmic reticulum were tested using adrenaline (1 nM and 1 µM) and ryanodine (10 µM). On the other hand, both aerobic metabolic scope and critical swimming speed were measured in fish challenged through a swimming-step protocol. In case of oil spills, dispersion-related impairments of these functions will be discussed in term of the animal fitness.

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**A5.79****Poster Session – Monday 29th June 2009****Ultrastructural study of the pallial reproductive tract of the protandric neogastropod *Coralliophila meyendorffii* (Gastropoda: Muricidae)**

Ma Jose Amor Perez (Universitat de Barcelona), Ma José Amor Perez (Universitat de Barcelona), Montserrat Ramón (Institut de Ciències del Mar (CSIC), Barcelona), Mercè Durfort (Universitat de Barcelona)

Within the framework of a more comprehensive study focusing on the evolutionary biology of Coralliophilinae, a highly specialized radiation of the muricid family whose species are anthozoan symbionts, the present work undertakes an ultrastructural study of the reproductive tract of the Mediterranean species *Coralliophila meyendorffii*. Within gastropods, reproductive characters are useful for unravelling phylogenetic relationships at different taxonomic levels. Previous studies on the anatomy of the pallial reproductive tract and on the spermatogenesis of Muricidae have revealed a wide variability among species in the histology and anatomy of the accessory reproductive glands and in sperm ultrastructure pointing to the potential use of these characters in phylogenetic studies. Nevertheless, for a correct interpretation of these characters, ontogenetic and ultrastructural studies are crucial. Preliminary results of the ultrastructure of the prostate and the capsule gland of *C. meyendorffii* are here presented and compared with the ultrastructure of the homologous glands of the muricine *Bolinus brandaris*. One of the most outstanding results are the differences among the species in the epithelial cells of the capsule gland. In *C. meyendorffii*, the epithelial cells lack secretory vesicles and Golgi apparatus, while in *B. brandaris* they are present suggesting that the epithelial cells in the latter species are involved in the elaboration of the final secretory product. It is here hypothesized that functional similarity in epithelial and glandular cells of the capsule gland might indicate a plesiomorphic state.

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#### A5.80

##### Poster Session – Monday 29th June 2009

##### Welfare and enrichment criteria for fish used in research

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Researchers working with animals are urged to strive towards treating animals as humanely as possible. The Animals Scientific

Procedures Act 1986 protects all living vertebrates used in scientific procedures in Great Britain. In 2007, 3.2 million scientific procedures were started which was a rise of 6% on 2006. The increased use of animals was in part due to a rise of numbers of fish. However, despite the fact that numbers of fish used in research is growing, guidelines for monitoring and reporting their welfare are meagre compared to policies available for mammals.

Researchers undertaking aquatic environmental protection work are recommended by the Home Office to promote animal welfare. Nevertheless, we have little or no evidence that suggested husbandry changes benefit concerned fish species, as most are derived from mammalian research. Some environmental enrichment changes proposed might even have negative effects, e.g. adding objects to fish tanks could cause aggression and stress. In addition, they may not be compatible with regulatory work, e.g. plastic objects could release endocrine-disrupting chemicals.

We aim to identify species requirements and investigate ways of providing environmental enrichment that are species-based and compatible with regulatory research. A variety of enrichment procedures, such as addition of novel objects to fish tanks will be tested for benefits for coldwater species commonly used in research. A suite of behavioural and physiological techniques will be used to investigate stress related biomarkers (such as hormonal balance and aggression). We will present some of our initial findings from our studies.

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