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SAT – CONSERVATION PHYSIOLOGY: ASSESSING AND FORECASTING THE RESPONSES OF ORGANISMS TO ENVIRONMENTAL CHANGE

SAT.1

09:00 Tuesday 30th June 2009

Conservation physiology: Assessing and forecasting the responses of organisms to environmental change

Craig E. Franklin (School of Biological Sciences, The University of Queensland)

Understanding and predicting how organisms respond to environmental change is becoming increasingly important as ecosystems are modified and threatened by human activity and rapid population growth. Effects of anthropogenic disturbance are wide-ranging and can influence all levels of biological organisation: from the genome, to biochemical and physiological function; to organismal performance, and to the maintenance of ecosystem services and biodiversity. The challenge ahead for biologists is to predict how organisms will respond and, if possible, adapt to rapid environmental change.

Conservation physiology explores the responses of organisms to anthropogenic threats and attempts to determine the ecophysiological constraints dictated by current conditions and future environmental change. It is an exciting emerging discipline that is gaining prominence in the literature as highlighted by a number of recent reviews (Carey, 2005; Wikelski and Cooke, 2006; Chown and Gaston, 2008). Underpinned by ecological and physiological theory, conservation physiology takes a multidisciplinary and integrative approach that encompasses both field and laboratory based research. It aims to determine and assess the proximate abiotic factors that impose fitness consequences upon the organisms as a result of anthropogenic threats and thus allows us to forecast the responses of organisms to environmental change. As such it will assist in determining the degree of threat to organisms and therefore help to set priority areas for conservation action.

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Chown, S.L. and Gaston, K.J. (2008) *Proc. R. Soc. B* 275, 1469–1478

Wikelski, M. and Cooke, S.J. (2006). *TREE* 21, 38–46.

Email Address for correspondence: c.franklin@uq.edu.au

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SAT.2

09:10 Tuesday 30th June 2009

Conservation physiology: Opportunities and challenges

Steven J. Cooke (Carleton University)

The recognition that physiological tools and knowledge have the potential to inform conservation and management decisions has led to the definition of the nascent discipline of “conservation physiology”. The premise is that physiology can yield insight into mechanisms associated with population declines and enable the quantification of organismal responses to different environmental changes. However, there remain a number of challenges that may retard the adoption of this approach or the realization of its full potential. For example, although it may be possible to identify a physiological basis for a reduction in individual fitness, there may be compensatory mechanisms at the population level that negate the individual-level problems. Clearly there is need for more research on how stress at the level of the individual can affect population-level processes. In addition, there is also an apparent disconnect between physiological information and conservation and management decisions, actions, and policy. How can we more effectively use data derived from physiological studies to inform policy? Using the more developed “conservation/animal behaviour” interface as a model, a template is presented for overcoming the apparent challenges that are limiting the potential of conservation physiology to aid in reversing the biodiversity crisis and to enable the sustainable management of natural resources.

Email Address for correspondence: Steven_Cooke@carleton.ca

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SAT.3

09:45 Tuesday 30th June 2009

Can within-individual plasticity buffer animals from climate variability?

Frank Seebacher (University of Sydney), Craig E. Franklin (University of Queensland)

Temperature has an acute thermodynamic effect on all biochemical reaction rates. This thermodynamic effect will constrain animal function and fitness if reaction rates become excessively high or low. Hence, animals respond to temperature variation behaviourally or by modulating reaction rates to minimise the thermodynamic effect. The time course of animal responses comprises within-individual plasticity (acclimation, acclimatisation) that may occur once during development or reversibly over periods of weeks, and genetic adaptation over many generations. The current human induced climate changes are very rapid so that in animals with relatively long generation times (>1 year) there are very few generations across which adaptive, genetic changes may occur. Hence, within-animal plasticity is likely to be the predominant mechanisms that can buffer animals from climate change. Thermal acclimation occurs across all vertebrate and many invertebrate taxa, and across all levels of organisation from growth and locomotion to transcriptional regulation of cellular functions. Understanding thermal acclimation is a necessary prerequisite to predict the potential effect of climate change on animal populations.

Email Address for correspondence: fseebach@bio.usyd.edu.au

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SAT.4

10:30 Tuesday 30th June 2009

Animal temperature limits and ecological relevance: Effects of size, activity and rates of change

Lloyd S. Peck (British Antarctic Survey), Simon Morley (British Antarctic Survey), Melody S. Clark (British Antarctic Survey)

Climate change is affecting species distributions and will increasingly do so. However, current understanding of individual and species survival capacities is poor. Knowledge of assemblage or community level effects is limited and the balance of mechanisms that are important over different time-scales is poorly described. Laboratory experiments on marine animals predominantly employ rates of change 10–100 000 times faster than climate induced oceanic warming. To address this failure we investigated differences in individual and species abilities to tolerate warming, and also how rate of warming affected survival. This study identifies community level effects of thermal biology by applying a multi-species, multi-trophic level approach to the analysis of temperature limits. Within species analyses of 14 species from 6 phyla showed smaller individuals survived to higher temperatures than large animals when temperatures were raised acutely. If this trend continues at slower warming rates, the early loss of larger individuals has marked consequences at the population level. Between species comparisons showed active species survived to higher temperatures than sessile or low activity groups. Thus active groups (e.g. predators) and juvenile or immature individuals should fare better in rapid warming scenarios. The rate of warming markedly affected temperature limits in a wide range of Antarctic marine species. Different species survived to temperatures of 8.3–17.6 °C when temperatures were raised by around 1 °C day⁻¹. However they only survived to temperatures between 4.0 °C and 12.3 °C when temperatures were raised by around 1–2 °C week⁻¹, and temperatures of only 1.0–6.0 °C were tolerated for acclimations over periods of months.

Email Address for correspondence: l.peck@bas.ac.uk

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SAT.5

10:50 Tuesday 30th June 2009

Assessing metabolic changes of the reindeer lichen *C. portentosa* to increasing environmental N inputs using metabolomic fingerprinting and profiling techniques

Sabine Freitag (University of the Highlands and Islands), Simon C. Thain (University of the Highlands and Islands), Angela H. Squier (University of the Highlands and Islands), Erika J. Hogan (The University of Nottingham), Peter D. Crittenden (The University of Nottingham)

Regional variation in wet N deposition in the British Isle is reflected in the total tissue N concentration of the common heathland lichen *Cladonia portentosa* (Hyvaerinen & Crittenden, 1998). The question remains, however, if and how metabolic pathways are altered within this symbiotic organism due to increasing wet N inputs. In this study we present data on the metabolic response of the lichen *C. portentosa* to an increasing gradient of atmospheric N inputs collected from 13 different sites across the UK. Initial metabolomic fingerprinting of *C. portentosa* was performed using Fourier-transform mid-infrared spectroscopy (FTIR). The soluble metabolome was subsequently studied by liquid chromatography-mass spectrometry (LC-MS). Data sets obtained from each analytical technique were correlated with atmospheric data (provided by the Centre of Ecology and Hydrology) including wet nitrogen deposition, nitrogen concentration and precipitation using multivariate modelling approaches such as principal component analysis (PCA) and partial least squares regression analysis (PLSR). Metabolic biomarker signatures were identified which showed good correlation particularly to the wet N deposition gradient. In addition a degree of differential parameterisation was observed in the modelled responses to each of the three environmental input parameters. The biological consequences for these findings will be discussed as well as the methodology used, which represents an effective integration of the complementary analytical techniques of FTIR and LC-MS as tools for environmental metabolomic studies.

Email Address for correspondence: Sabine.Freitag@thurso.uhi.ac.uk

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SAT.6

11:05 Tuesday 30th June 2009

First evidence of a potential bactericidal effect due to the phenoloxidase system in the Pacific oyster *Crassostrea gigas*

Luna Acosta Andrea (University of La Rochelle—LIENSs laboratory UMR 6250 CNRS), Pommier Mylène (University of La Rochelle—LIENSs laboratory UMR 6250 CNRS), Thomas-Guyon Hélène (University of La Rochelle—LIENSs laboratory UMR 6250 CNRS), Haffner Philippe (IFREMER—LGP laboratory La Tremblade)

Increasing mortality events of *Crassostrea gigas* have been observed during the last decade and it has been suggested that this could be due to an impairment of immune defences. However, little is known on immune defences in this economically and ecologically important organism. Although the prophenoloxidase system is known to be implicated in immune defences in several invertebrates, has been detected on the haemolymph of *C. gigas* and has been proposed as an interesting biomarker for immunological studies, experimental evidence is lacking that the prophenoloxidase system participates in immune defences against microorganisms infecting this particular host. In the present study, haemolymphatic acellular fraction (HAF) and haemocyte lysate supernatant (HLS) were analysed for their

ability to inhibit *in vitro* the growth of two major *C. gigas* bacterial pathogens associated to summer mortality phenomenon of cupped oyster in France: *Vibrio aestuarianus* and *Vibrio splendidus*. Interestingly, in the presence of l-Dopa phenoloxidase substrate, HLS exhibited a bactericidal effect at least on one of the two studied pathogenic *V. aestuarianus* contrary to HAF. This is the first time that (i) a bactericidal effect potentially involving phenoloxidase system in *C. gigas* is demonstrated and that (ii) an *in vitro* model of experimental immune action of the phenoloxidase system is developed. These results could be of particular interest for immunological and immunotoxicological studies in *C. gigas* and help to evaluate the impacts of human-induced environmental change on this organism. Use of the appropriate inhibitors of phenoloxidase system to assess the specificity of the observed bacterial growth inhibition is discussed.

Email Address for correspondence: aluna1508@yahoo.com

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SAT.7

11:20 Tuesday 30th June 2009

Thermal tolerance of various larval stages of the Southern kelp crab *Taliepus dentatus*

Daniela Storch (Alfred-Wegener Institute for Polar and Marine Research Bremerhaven, Germany), Miriam Fernández (Estación Costera de Investigaciones Marinas Pontificia Universidad Católica de Chile Santiago), Sergio Navarrete (Estación Costera de Investigaciones Marinas Pontificia Universidad Católica de Chile Santiago), Hans-O. Poertner (Alfred-Wegener Institute for Polar and Marine Research)

Temperature variability can influence patterns of larval distribution, development and recruitment through its effects on larval physiology. A mechanistic understanding of physiological responses that affect larval survival is crucial to understand ecological patterns. We therefore investigated how thermal tolerance might contribute to setting distribution limits and/or recruitment of crab larvae. The thermal tolerance windows for zoea I, II and megalopa of *Taliepus dentatus* were determined by measuring pleopod beat rates, active metabolic rates and heartbeat to identify the most sensitive larval stage in relation to the environmental temperature of the species. Zoea I showed the broadest and megalopae the narrowest tolerance window.

The thermal tolerance windows for megalopa were further specified by lower and upper pejus (Tp) and critical (Tc) temperatures. Tps limiting the temperature range where aerobic scope is maximal and functioning of the organism is unrestrained, were derived from pleopod beating. Tcs indicating the transition from aerobic to anaerobic metabolism were determined as those limits, where standard metabolic rate leaves its exponential temperature dependency at extreme temperatures. Optimum performance in megalopae was limited to a narrow window between Tps of 11 and 15 °C, while the Tc envelope ranged between 7 and 19 °C. The narrow optimum range of megalopae suggests that thermal preferences and associated behaviour may be critical to control the position in the water column thus contributing to predator avoidance, settling to the adult habitat and recruitment. We hypothesize that inter-annual variability in field exposures to temperatures beyond Tps and/or Tcs can influence recruitment of *T. dentatus* through physiological limitations.

Email Address for correspondence: Daniela.Storch@awi.de

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SAT.8

11:35 Tuesday 30th June 2009

The use of multi-factorial experimental studies in determining the causes of amphibian declines

Lesley A. Alton (The University of Queensland), Robbie S. Wilson (The University of Queensland), Craig E. Franklin (The University of Queensland)

Amphibian declines are a prominent part of the global biodiversity crisis and have received special consideration because they have occurred relatively recently, on a global scale, and in seemingly pristine habitats where no obvious anthropogenic cause is apparent. Determining the cause of amphibian declines is therefore one of the greatest challenges currently facing conservation biologists. Although several causes for declines have been implicated, continuing research has made it clear that there is no simple explanation. Consequently, it has been hypothesised that complex interactions between multiple environmental stressors, particularly those associated with global change, may be responsible. The challenge associated with multiple stressors is that their combined effect cannot be predicted from single-stressor studies. Multi-factorial studies have therefore been identified as a key area of research that is needed to disentangle the underlying mechanisms behind global amphibian declines. Using this approach with a controlled laboratory experiment we examined the interactive effects of ultraviolet-B radiation (UV-B) and non-lethal predatory chemical cues on the survival and morphology of *Limnodynastes peronii* tadpoles. We show that UV-B and predatory chemical cues interact synergistically to enhance mortality above the additive effects of the independent stressors, and that exposure to UV-B affects the ability of tadpoles to morphologically respond to predatory chemical cues (i.e. predator-induced phenotypic plasticity), which has implications for their survival in a predator environment. This research highlights the importance of using such experimental approaches to assess potential causes for species declines.

Email Address for correspondence: l.alton@uq.edu.au

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SAT.9

11:50 Tuesday 30th June 2009

Could daily torpor be modulated in response to environmental challenges?

Cindy Canale (CNRS/MNHN)

As ecosystems undergo global changes, there is an increasing interest on understanding how organisms respond to changing environments. In the tropics, climate change results in more extreme events. The induced food shortage may impact the energetic trade-off between metabolic demands. We tested with a food restriction experiment, how captive Grey Mouse Lemurs (*Microcebus murinus*) modify torpor-based energy savings in response to contrasted regimes of food availability, and to an immune challenge.

Twelve individuals were fed *ad libitum*, and 12 were exposed to a 40% caloric restriction during 11 weeks. Afterwards, six of each group were exposed to a 2-week 80% caloric restriction. Two weeks later, six individuals per group (ALI and CRI) received a challenge that activates both innate and acquired immunity. Energetic modulations were characterised by changes in daily torpor (body temperature).

Results. Daily torpor was increased by chronic caloric restriction, with a relatively small effect of acute restriction. All immune-

challenged individuals exhibited a pyrogenic response, and skipped diurnal torpor. The pyrogenic response was stronger in CRI than in ALI, but CRI individuals returned more rapidly to deep torpor than ALI individuals.

During 'bad' years and extreme events, individuals would increase the amount of energy savings by increasing the length and the depth of torpor. When exposed to a pathogen, the acute response of undernourished individuals would be energetically constrained. Our results suggest that facultative, heterothermic organisms may overcome climate-driven, sudden changes in food availability by modulating their energy savings. However, this may have an immune cost.

Email Address for correspondence: canale@mnhn.fr

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SAT.10

12:05 Tuesday 30th June 2009

The "Dammed" – Hatchling bimodal-respiring turtles

Craig E. Franklin (School of Biological Sciences, The University of Queensland), Natalie J. Clark (School of Biological Sciences, The University of Queensland)

River damming results in significant changes to environmental water conditions including the loss of pool-rifle sequences, reduced water flow, alterations in temperature profiles, increased water depth and decreased oxygen levels. The impacts of these physio-chemical changes upon freshwater turtles are poorly known. Australia has a diverse freshwater turtle fauna, with a number of species being able to respire bimodally; acquiring oxygen from the water cutaneously, via the buccopharynx and/or cloacal bursae. In some species, aquatic respiration can account for more than 70% of the total oxygen requirements and this translates into longer dive times.

Body size can strongly influence physiological processes and in turn influence behaviours and ecological performance. For diving animals, the general trend is that dive duration decreases with a decrease in body size, chiefly as a consequence of smaller animals having a higher mass specific metabolic rate. Aquatic respiration has the potential to change this relationship and this in turn could have implications to hatchling turtles living in disturbed habitats. Here, we highlight the necessity to consider body size as an important variable when assessing the impact of habitat change.

Email Address for correspondence: c.franklin@uq.edu.au

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SAT.11

13:30 Tuesday 30th June 2009

Ecosystem impacts of climate change and ocean acidification: A case for "global" conservation physiology

Hans O. Pörtner (Alfred-Wegener-Institute)

Climate change causes ocean warming and acidification on global scales. In contrast to well established effects of warming, evidence for the effects of rising carbon dioxide (CO₂) on marine ecosystems is only just emerging. However, future scenarios indicate threats to marine life through combinations of rising CO₂, warming and more frequent hypoxia events. For realistic future climate effect scenarios, key physiological mechanisms and their responses to combined stressors require identification. These are physiological mechanisms

which define species performance, including their capacity to interact, e.g. in food webs (1). Many ecosystem changes likely occur when temperature drifts beyond species-specific limits and causes a shift in phenology or fitness. High sensitivity to elevated CO₂ levels may involve a low capacity for acid–base regulation, as seen in lower marine invertebrates (2). The disturbed extracellular acid–base status affects processes involved in growth, calcification, neural functions, blood gas transport and behavioural capacities (2). Current evidence indicates elevated sensitivity to elevated CO₂ levels towards the extremes of thermal windows (3). The ultimate consequence may be a narrowing of thermal tolerance windows and associated ranges of geographical distribution and of the performance at ecosystem level. Thus, CO₂ may exacerbate warming effects on marine ecosystems. Future research will have to test these concepts under realistic climate and ocean acidification scenarios and in various marine ecosystems between the tropics and the poles.

1. Science 322: 690–692 (2008)

2. Marine Ecology Progress Series 373: 203–217 (2008)

3. Journal of Thermal Biology 32: 144–151 (2007)

Email Address for correspondence: hans.poertner@awi.de

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SAT.12

14:05 Tuesday 30th June 2009

Physiological performance of Baltic Sea blue mussels, *Mytilus edulis*, under CO₂ induced stress: Cardiac performance, activity and filtration rate

Julia Saphörster (IFM-GEOMAR Kiel, Germany), Jörn Thomsen (IFM-GEOMAR Kiel, Germany), Frank Melzner (IFM-GEOMAR Kiel, Germany)

Anthropogenic CO₂ emissions will lead to an increased ocean pCO₂ level of possibly 1900 ppm in the year 2300. Previous studies depicted that bivalves might react very sensitively towards increased seawater pCO₂ with decreased rates of calcification, metabolism and growth. In order to assess the impacts of simulated ocean acidification on a Baltic Sea *M. edulis* population, we simultaneously monitored heart rate variability, filtration and activity patterns, as well as extracellular acid base status and calcification.

Adult mussels were kept in flow through aquaria for two weeks at six different CO₂ concentrations between 380 ppm and 4000 ppm. Mussels were fed continuously with a mixed phytoplankton concentrate. Measurements of heart rate, heart rate variability, valve and siphon opening revealed no effect of pCO₂ on cardiac performance and activity, even though significant extracellular acid–base disturbances and decreases in calcification rate were recorded. Filtration rates, on the other hand, were significantly depressed at 4000 ppm. In an ongoing study, mussels are continuously being fed with *Rhodomonas* sp. at a concentration of 1000–4000 cells/ml in order to study filtration efficiency in more detail.

Physiological changes might not only affect the mussel's health but the whole ecosystem, since filter feeding bivalves act as ecosystem engineers, not only changing the inorganic nutrient pool available to phytoplankton, but also producing faeces and pseudofaeces, which can reduce erosion by 10-fold in some areas. Being the dominant benthic invertebrate, dense mussel beds even provide an important settling ground for various other organisms.

Email Address for correspondence: jsaphoerster@ifm-geomar.de

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SAT.13**14:20 Tuesday 30th June 2009****The climate for migration by Christmas Island red crabs: A dangerous dependence on December downpours?**

Steve Morris (University of Bristol), UtePostel (University of Bristol), Simon G. Webster (Bangor University)

The terrestrial Christmas Island red crab, *Gecarocidea natalis*, exhibits a seasonal breeding migration to the ocean, synchronized with the monsoon rains. The Australian Government recognizes this phenomenon as 'one of the natural wonders of the world' and reports that the ecosystem of Christmas Island may be impacted by climate change. There is growing opinion that this Island's ecology is on the cusp of disaster, possibly due to disturbed rainfall patterns and from effects of anthropogenic activities. We investigated environmental and behavioural influences on hormonal regulation of energy provision to migrating crabs against the backdrop of seasonal rainfall and activity, within the context of limitations on fuel storage and usage and the consequences of 'rushed' or 'protracted' migrations. Crustacean Hyperglycaemic Hormone (CHH) mediates the mobilization of glucose in migrating crabs, promoting a diurnal hyperglycaemia. We show that elevated blood glucose feeds-back to inhibit CHH release. Burrowing, fighting and mating activities deplete the energy stores of male crabs which have low blood glucose, but CHH elevated in an ineffective attempt to stimulate a hyperglycaemia, lipid metabolism is activated. Prolonged migratory activity can place the crabs in situations of severe energy depletion. Metabolic evidence from dry season crabs for a negative feedback on hexokinase suggests that glycolysis is markedly slowed which may allow for energy storage if humidity is above the 85% needed to allow foraging. These data are assessed generally in light of the apparently progressive delay in the arrival of the seasonal monsoon and the propensity for failed migrations.

Email Address for correspondence: Steve.Morris@bristol.ac.uk

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SAT.14**14:40 Tuesday 30th June 2009****A cheap and simple method for assessing the condition of a reptile population**

Hamish A. Campbell (The University of Queensland)

Sustainable harvesting of wild crocodylians remains a hot topic within South America. We undertook a study within the Pantanal wetlands, Brazil, to enable more pragmatic management of the inhabiting *Caiman crocodylus yacare*. Animal movements were observed by radio tracking throughout the year, and physical condition assessed by body measurements and the relative concentration of metabolites recorded in the blood (glucose, triglycerides, β -hydroxy-butyrate & uric acid). During the wet season the caiman remained relatively stationary within an area and were well fed. As the dry season progressed the animals congregated in large numbers around remaining ponds, and feeding rate was reduced considerably. Subordinate males were forced out of the ponds and were found starving in dry grassland, many were so emaciated that recovery seemed unlikely. We conclude that the *C. yacare* population within our study region was probably at carrying capacity for the quantity of surface water, and many adult males were effectively redundant from the breeding population. Future conservation management should focus on the preservation or even creation of permanent ponds,

which appear to be critical for the species survival throughout the dry season.

The methodology used in this study proved very effective for sampling a population of reptiles at an isolated location. It was completed on a small budget and could be efficiently carried out by students and volunteers.

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SAT.15**15:00 Tuesday 30th June 2009****Visual, behavioural, and energetic determinants of the effect of climate change on great cormorants in Greenland**

Craig R. White (The University of Queensland), David Grémillet (Centre National de la Recherche Scientifique), Patrick J. Butler (The University of Birmingham), Jonathan A. Green (The University of Liverpool), David Boertmann (National Environmental Research Institute University of Aarhus), Graham R. Martin (The University of Birmingham)

The ways in which animals respond to climate change are likely to be seen most clearly in populations living at the limits of their distribution. In the present study, we combine field and laboratory measurements of great cormorants *Phalacrocorax carbo* to understand how populations in Greenland, at the northern limit of the range of the species, are likely to respond to a warming Arctic. In the laboratory, we measured the visual acuity and energetics of captive birds; in the field, we measured daily energy expenditure, diel patterns of behaviour, and foraging efficiency. We show that these cormorants are remarkably efficient predators, that their daily energy expenditure is unexpectedly low, and that population growth rates are positively correlated with sea surface temperature. These observations suggest that these cormorants are well suited to existence within the Arctic Circle, but that they might nevertheless benefit from a warming Arctic. However, we also show that cormorants have poor visual acuity when in water, that their acuity declines precipitously at low levels of ambient illumination, and that diving is almost entirely restricted to daylight hours, except during the shortest midwinter days. Thus, while it might be expected that retreating sea ice could increase the breeding and wintering range of great cormorants in Greenland, we argue that sensory constraints on foraging behaviour are likely to restrict the wintering range of this visually-guided predator to its present locations south of the Arctic Circle where ambient light levels are sufficiently high to allow foraging.

Email Address for correspondence: craig.white@uq.edu.au

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SAT.16**15:40 Tuesday 30th June 2009****Hemoglobin polymerization in marine fishes: An adaptation to environmental stress?**

Ione Hunt von Herbing (University of North Texas)

Investigations of the phenomenon of hemoglobin (Hb) polymerization in red blood cells in several fish species under low O₂ and pH conditions, suggest it may be a unique example of Hb phenotypic plasticity, and characteristic of species that inhabit extreme or variable environments. Hb polymerization, is also

termed gelation and/or sickling, and its physiological characteristics, and importance to fish physiology is unknown. Data from 33 species were gathered on the frequency and physiology of Hb polymerization from Arctic, boreal and subtropical seas, but only 13 species, including those in the Gadiformes (cods), and Batrachoidiformes (toadfishes), exhibited strong Hb polymerization. In these species, purification of Hb determined that polymerization is an intrinsic property of the Hb, and not dependent on the surrounding intracellular matrix. TEM sections from juvenile Atlantic cod (*Gadus morhua*) after exposure to low O₂, recorded polymerized Hb within muscle arteries, showing that it occurs *in vivo*, as well as *in vitro*. Sequence analysis of globin cDNAs of 3 gadid species provided models of cod Hb constructed by homology modeling of the primary Hb sequence and identified at least three prominent amino acid substitutions. Two of these result in external cysteines that may induce polymerization by forming interchain thiol bonds, which may serve an antioxidant role against reactive oxygen species, often produced during aerobic metabolism in environments with fluctuating conditions. Results from this work may serve as a future tool for conservation physiology, and ultimately contribute to our understanding of how resilient or weak populations are to global environmental stress.

Email Address for correspondence: vonherbing@unt.edu

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SAT.17

15:55 Tuesday 30th June 2009

The life of the dammed – development of the stress axis in the lake sturgeon, *Acipenser fulvescens*

Sadaf Zubair (University of Manitoba), Stephan J. Peake (University of New Brunswick), Gary Anderson (University of Manitoba)

Once abundant in its natural range of the Great Lakes and Hudson Bay drainage basins; the lake sturgeon, *Acipenser fulvescens*, is currently listed as threatened in Canada and the United States. Significant efforts are underway to determine key life history requirements and physiological needs for these fish in the face of a changing global environment coupled with the continuing and increasing demand for sustainable energy sources such as hydro power. In the present study we examined the relationship between substrate type and the development of the stress axis in lake sturgeon. Fertilised eggs were incubated on two substrate types, gravel and slate. Post hatch, the slate substrate was removed and all fish were then allowed to develop on a gravel substrate until the onset of exogenous feeding. Percent survival to the onset of exogenous feeding was $8.71 \pm 1.0\%$ for gravel and $9.96 \pm 1.2\%$ for slate and was not significantly different between treatments. Baseline values for whole body cortisol in fish allowed to develop in gravel were $6.56 \pm 0.7 \text{ ng g}^{-1}$ wet body mass (bm), with a maximum response at 5 min post stress of $11.23 \pm 0.95 \text{ ng g}^{-1}$ wet bm. Baseline values for whole body cortisol in fish allowed to develop on slate were $8.89 \pm 1.05 \text{ ng g}^{-1}$ wet bm with a maximum response at 10 min post stress of $14.15 \pm 1.78 \text{ ng g}^{-1}$ wet bm. These findings have implications for hatchery rearing of lake sturgeon for re-stocking programs in rivers where populations are particularly low.

Email Address for correspondence: andersow@cc.umanitoba.ca

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SAT.18

16:10 Tuesday 30th June 2009

A comprehensive examination of the effects of chronic hypoxia on the cardiorespiratory physiology of Atlantic cod (*Gadus morhua*)

Lene H. Petersen (Ocean Sciences Centre Memorial University), Kurt Gamperl (Ocean Sciences Centre Memorial University)

Chronic hypoxia is an increasing concern in coastal and marine environments. However, the physiological effects of chronic hypoxia on fishes, and their capacity to adapt to such challenges, are poorly understood. Thus, we acclimated Atlantic cod to either 40 or 100% air saturation for 6–12 weeks at 10 °C, and performed *in vivo*, *in situ*, and *in vitro* experiments to examine the effects of chronic hypoxic exposure on various aspects of performance and cardiorespiratory function. These experiments showed that: 1) hypoxic acclimation does not alleviate the negative effects of acute hypoxia on the cod's metabolic capacity, maximum cardiac function (*Q*) or swimming performance (~30% decrease in *U*_{crit}); 2) while max. *in vivo* *Q* during both normoxia and acute hypoxia is reduced in chronically hypoxic cod, this does not compromise MO₂ because of a concomitant increase in O₂ extraction efficiency; 3) hypoxic-acclimation diminished the already low sensitivity of the cod heart to adrenaline, but that cod chronically exposed to hypoxia have an enhanced capacity to release plasma catecholamines during severe hypoxia; 4) although cod acclimated to hypoxia have higher blood [haemoglobin] and haematocrit levels, only minor changes in haemoglobin–oxygen binding characteristics were noted; and 5) while hypoxic-acclimated cod had a lower critical oxygen tension (*P*_{crit}), this did not improve their ability to tolerate severe hypoxia. These results provide important insights into how fish cardiorespiratory physiology is impacted by short-term and prolonged hypoxia, and further highlight the tremendous capacity of some aspects of the fish cardiorespiratory system to deal with environmental challenges.

Email Address for correspondence: lh0011@unt.edu

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SAT.19

16:25 Tuesday 30th June 2009

Histopathology and oxidative stress in barbel (*Barbus bocagei*) of the Vizela River (North Portugal), a first approach

Ana M. Coimbra (CITAB—Centro de Investigação e Tecnologias Agroambientais e Biológicas), António Fontainhas-Fernandes (CITAB—Centro de Investigação e Tecnologias Agroambientais e Biológicas), Maria M. Oliveira (CQVR—Centro de Química-Vila Real), João Carrola (CITAB—Centro de Investigação e Tecnologias Agroambientais e Biológicas), Francisco Peixoto (CECAV—Centro de Ciência Animal e Veterinária)

Vizela River flows through area of the north of Portugal, where textile industry is predominant. This discharges high amounts of water with contaminants, especially products used during the dyeing process.

This study was designed as a first approach to evaluate the health of barbel from Vizela River, using liver histopathology and oxidative stress as biomarkers of exposure. Liver was chosen due to its role in contaminants accumulation and metabolism, which can induce both structural and functional alterations, normally associated with levels of water contamination.

Barbel were captured in the Vizela River and liver samples were collected for histology. Lipid peroxidation and hepatic activities of superoxide dismutase, catalase, glutathione *S*-transferase, glutathione reductase, glucose 6-phosphate dehydrogenase, xantine oxidase and

amounts of reduced glutathione were measured. Barbel collected in an unpolluted site was used as reference.

Liver histology showed the presence of several hepatic changes: macrophage aggregates, lymphocytic focus, unspecific granulomas, vacuolization and necrosis. A score of the lesions revealed a significant increase in macrophage aggregates and lymphocytic focus presence in Vizela River barbel.

Lipid peroxidation was higher in the Vizela River barbel. Likewise, activities of superoxide dismutase, catalase, glutathione S-transferase, glutathione reductase, glucose and 6-phosphate dehydrogenase were increased. However, no differences were observed for xantine oxidase activity and reduced glutathione amounts.

The biomarkers of exposure tested in Vizela River barbell proved to be efficient, since liver histology and oxidative stress parameters studied significantly differ from reference barbel, showing that the textile industry effluents interfere with Vizela River fish health.

Email Address for correspondence: acoimbra@utad.pt

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SAT.20

16:40 Tuesday 30th June 2009

Conservation physiology of fishes of the Salton Sea, California

Colin J. Brauner (Zoology, University of British Columbia), Brian A. Sardella (Biology, Eastern Washington University)

The Salton Sea in southeastern California, USA, is a 1000 km² lake that was created in 1905 when the Colorado River was accidentally diverted into a desert valley. Due to no outflow, continued water input, and high evaporative water loss, the salinity has increased from river water to a current value of 48 g/l and continues to increase at 0.3 g/l annually. Many fish species have historically inhabited the sea, however, the California Mozambique tilapia is currently the most dominant species. These tilapia can tolerate salinities up to 65 g/l at 25 °C with minimal effects on osmoregulatory status. However, a reduction in temperature to 15 °C or an increase to 35 °C (both within the range of temperatures observed in the Salton Sea) greatly reduces the salinity tolerance of this species; the former through massive impairment of gill Na⁺, K⁺ ATPase activity, and the latter likely due to an osmorepiratory compromise. Our data indicate that the seasonal winter kills, that can represent over 80% of the tilapia population, are likely associated with a direct effect of temperature on salinity tolerance at the current salinity of the Salton Sea. The sea represents a large proportion of the wetland for fish and wildlife in the region and is an important stopping point in the Pacific flyway of migratory birds. Environmental managers are considering scenarios to prevent further increases in the salinity of the sea to maintain the current fishery that appears to be on the verge of its salinity tolerance.

Email Address for correspondence: brauner@zoology.ubc.ca

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SAT.21

Poster Session – Tuesday 30th June 2009

Are populations in the edge of their distribution range more stressed? A non-invasive field evaluation of stress in the European badger (*Meles meles*) along an environmental gradient in the Iberian Peninsula: Implications for global change

Gema Escribano (Universidad Rey Juan Carlos), Emilio Virgós (Universidad Rey Juan Carlos), Isabel Barja (Universidad Autónoma de

Madrid), Carlos Lara Romero (Universidad Rey Juan Carlos), Mariano Recio (Universidad Rey Juan Carlos)

Global change is menacing biodiversity, new multidisciplinary approaches as Conservation Physiology and Macrophysiology are required in the study of how global change influences biodiversity. These new disciplines focus in new and higher accuracy methods to know how individual variables are related with environmental gradients and new threats.

Our aim is to evaluate the stress levels of European badgers according to an environmental gradient in the Iberian Peninsula, from temperate to arid areas. We hypothesized an increase in the stress levels according to the environmental gradient from the North to the South of the Iberian Peninsula based on the presumed best suitability for badgers in the temperate areas. In the other side, maximum levels of stress in the arid South were predicted based on the low suitability of these areas for badgers, which are far away from their 'ecological' optimum (edge of the distribution).

We collected faeces of European badgers in different populations along the environmental gradient established. From faeces we obtained stress levels (cortisol) with a non invasive immunoassay (ELISA). Sexual hormones and population density were measured as they act as potential confounding factors.

Our results do not confirm our hypothesis because stress levels were maximum in the North area and minimum in the Centre and the South area. Density and sexual activity cannot explain these biases from our predictions. We can suggest that badgers of the central area will be the best prepared to cope with global change.

Email Address for correspondence: gemita123@hotmail.com

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SAT.22

Poster Session – Tuesday 30th June 2009

Is density a good measure of habitat quality? A macrophysiological test with Eurasian badgers in Spain

Emilio Virgós (Universidad Rey Juan Carlos), Escribano Gema (Universidad Rey Juan Carlos), Carlos Lara (Universidad Rey Juan Carlos), Isabel Barja (Universidad Autónoma de Madrid)

Conservation of species is largely based on the concept of habitat quality. This is mainly described through changes in the abundance of species. We only have minor evidences about the relationships between habitat quality and abundance. Physiological indices related to individual fitness can provide a tool to deep in the interplay between the environment and how it is experienced by individuals and populations.

We used a macrophysiological approach to test the relationship between the abundance of badgers (*Meles meles*) and an index of individual well-being, their levels of cortisol (stress indicator). We tested this relationship in three populations located along a wide environmental gradient in the Iberian Peninsula from the North (presumed good conditions) to the South (presumed bad conditions).

Population density follows the predicted pattern: higher abundances in the north and centre of the Peninsula. In contrast, the a priori good areas in the north showed the highest levels of stress measured by cortisol. So, badger density and cortisol levels reflect different patterns of habitat quality.

Although our study is based in not abundant populations, we cannot conclude that large populations in the North are in better habitats than in the South or centre of the Peninsula. Therefore, density can be a misleading indicator of habitat quality when is not

filtered by more mechanistic (physiological) variables. Conservation of populations need both recognize the importance of density (probability of extinction) and some measure of the fitness of individuals.

Email Address for correspondence: emilio.virgos@urjc.es

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SAT.23

Poster Session – Tuesday 30th June 2009

Evaluating adrenal activity and effects related with the collection and conservation of faecal samples to understand the physiological stress responses of wild European badgers

Carlos Lara (Universidad Rey Juan Carlos), Isabel Barja (Universidad Autónoma de Madrid), Emilio Virgós (Universidad Rey Juan Carlos), Gema Escribano (Universidad Rey Juan Carlos)

For conservation strategies to be successful, it is important to understand the physiological stress responses of animals to the changes in their environment. Due to the increasing demand of methods to quantify adrenal activity in response to stressors in wild animals, in this study we evaluated if ACTH stimulation result in an increase of faecal cortisol levels in European badgers. Also, we carry out several experiments to evaluate if the conservation time and the collection of faecal samples affect to the cortisol levels. The faecal samples were collected from five captive European badgers and the quantification of faecal glucocorticoids was conducted by means of enzyme immunoassay. The faecal cortisol levels increased 1–2 days after ACTH injection (764.4 ng/g) and dropped to pre-treatment levels (474.5 ng/g) 3 days after ACTH stimulation. The cortisol concentrations varied for the same scat, being significantly higher when the samples were collected from the interior (348.9 ng/g) and surface (308. ng/g) of the scats that when those were homogenized (279.7 ng/g). Also, the faecal cortisol levels diminished significantly when the samples remained frozen during more time (1 month: 461.4 ng/g, 4 months: 175.7 ng/g, 7 months: 214.3 ng/g). However, the time lapsed from the deposition until the collection of the faecal samples did not affect to the cortisol levels. The measurement of faecal cortisol is a suitable method for the non-invasive evaluation of adrenocortical activity in European badgers. However, some aspects related with the collection and conservation of the faecal samples should be considered in these studies.

Email Address for correspondence: c.lara@hotmail.com

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SAT.24

Poster Session – Tuesday 30th June 2009

The effect of copper toxicity in apoptotic response and cell proliferation, in gills of *Oreochromis niloticus*

Sandra M. Monteiro (Center for the Research and Technology for Agro-Environmental and Biological Sciences), Nuno M.S. Dos Santos (Institute for Molecular and Cell Biology), Margarida Calejo (University of Porto), António Fontainhas-Fernandes (Center for the Research and Technology for Agro-Environmental and Biological Sciences), Mário Sousa (ICBAS, University of Porto)

Although copper is an essential element, it may become toxic when its concentration exceeds certain natural levels. Some *in vitro* studies suggest that copper may induce apoptosis triggering the activation of caspase-3, a central effector of apoptosis. However, the precise mechanism of copper-induced apoptotic cell death in fish is still unclear, even less in *Oreochromis niloticus* where no caspase genes have been reported. Accordingly, the present study aimed to assess the *in vivo* copper toxicity in apoptosis induction in *O. niloticus* gill, simultaneously contributing to elucidate the mechanism of copper induced apoptosis. Caspase-3 gene was partially sequenced (EU887950) and, after *in vivo* exposure to 40 and 400 $\mu\text{g L}^{-1}$ of copper, its expression was evaluated by real time-PCR. Apoptosis was also evaluated by TUNEL and cell proliferation identified using an antibody against proliferating cell nuclear antigen. The copper concentrations used failed to induce the upregulation of caspase-3 gene in tilapia gill. Additionally, the determination of the relative volumes of activated caspase-3 immune-reactive cells and of TUNEL positive cells showed that there were no significant differences between control and exposed fish. Moreover the immune-positive cells relative volume presented a slight decrease in filament epithelium and a no significant increase in lamellae after copper exposure. Thus, the overall results did not show indicia of apoptosis induction by copper in *O. niloticus* gill, neither through the enzymatic activation of the already existing caspase-3, nor through a caspase independent pathway. However, an increase in the volumetric density of epithelial proliferating cells was observed, suggesting a dose-dependent repair response.

Email Address for correspondence: smonteir@utad.pt

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