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### A11–GENERAL ANIMAL BIOLOGY

Organised by Tobias Wang (Aarhus, Denmark) and David McKenzie (DIFRES, Denmark)

#### A11.1

##### When, how, why and how often: Evolution of the Bohr effect in vertebrate haemoglobins

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The Bohr effect, which can be most generally defined as the influence of pH on the oxygen binding affinity of proteins, is a common feature of respiratory pigments, ranging from the haemocyanins of molluscs and crustaceans to the haemoglobins of vertebrates. Its physiological role is generally seen in the facilitation of oxygen release from respiratory pigments during tissue acidosis. The magnitude of the effect can be influenced by a multitude of factors such as temperature, carbon dioxide, chloride ions, organic phosphates and the investigated pH range. Here we present data on the maximal alkaline Bohr effect in haemoglobins from a large number of species covering all vertebrate classes, obtained at physiological temperatures in the presence of 100 mM chloride ions and the absence of carbon dioxide and organic phosphates. This data together with literature data obtained under comparable conditions is used to reconstruct the evolution of the Bohr effect on a vertebrate phylogeny [1]. Results suggest that the Bohr effect may have evolved as many as three times independently during vertebrate evolution, namely in agnathans, ray-finned fishes and amniotes. This is consistent with recent reports of distinct differences in the molecular mechanism of the Bohr effect between mammalian and teleost fish haemoglobins, and with the elucidation of yet another, entirely different molecular mechanism of the Bohr effect in agnathan haemoglobin.

Keywords: Bohr effect, Evolution, Oxygen transport, Haemoglobin, Vertebrates

I. M. Berenbrink, P. Koldkjaer, O. Kepp and A.R. Cossins (2005). Evolution of oxygen secretion in fishes and the emergence of a complex physiological system. *Science* 307, 1752–1757.

#### A11.3

##### The impact of high CO<sub>2</sub> concentrations on energy metabolism of Antarctic eelpout *Pachycara brachycephalum*

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Disposal of anthropogenic CO<sub>2</sub> to the deep sea and its impact on marine fauna is currently under debate in the context of strategies to mitigate global warming. We investigated the effects of CO<sub>2</sub> exposure on energy metabolism and acid–base regulation of the benthic Antarctic eelpout *Pachycara brachycephalum*, as a model for deep sea fish. Ventilation and oxygen consumption measurements were combined with in vivo <sup>31</sup>P-NMR spectroscopy of Antarctic eelpout under control conditions, during 74 hours of exposure to 1% CO<sub>2</sub> (hypercapnia) and after return to normocapnic conditions. Ventilation rates of *P. brachycephalum* increased three-fold, while oxygen consumption rose two-fold during fifteen hours of hypercapnia. Ventilation and respiration rates remained elevated during the whole hypercapnic period and were down regulated during recovery. As a result of respiratory acidosis white muscle intracellular pH decreased initially and returned to control values within 20 hours of CO<sub>2</sub> exposure. An overcompensation of pHi disturbance could be observed during the first 20 hours of normocapnic recovery with a subsequent return to control values. Our data indicate a permanent increase in energy turnover under the effect of CO<sub>2</sub>, due to an increase in ventilation costs. Previous studies have demonstrated a decrease in growth rates of fish under CO<sub>2</sub> (Crocker and Cech, 1996). Taken together, our findings may suggest that elevated ventilation costs under elevated CO<sub>2</sub> levels cause unfavourable shifts in energy budgets, with a reduction in growth and reproduction and, thereby, reduced fitness of a species. Keywords: Zoarcidae, CO<sub>2</sub>-disposal, <sup>31</sup>P-NMR Spectroscopy, Energy metabolism, Acid–base regulation Crocker C.E., Cech J.J. (1996). *Aquaculture* 147, 293–299.

#### A11.4

##### Adrenergic control of e–c coupling in fish cardiac myocytes

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Calcium flux across the sarcolemmal membrane through the L-type Calcium channel ( $I_{Ca}$ ) of isolated trout myocytes is dramatically increased by adrenergic stimulation. We have previously shown that the increase in  $I_{Ca}$  upon adrenergic stimulation is acutely temperature-dependent (1). The aim of the present study was to investigate which step(s) in the adrenergic signalling cascade are responsible for this

The objective of this study was to determine the effects of hypoxia on the number (receptor density,  $B_{max}$ ) and affinity ( $K_d$ ) of  $\beta$ -adrenoreceptors in ventricular micropunches by incubating the cardiac tissue with a tritiated  $\beta$ -antagonist ( $[^3H]CGP-12177$ ). Eggs from a fast-growing broiler chicken strain were incubated in normoxia (21%  $O_2$ ) and chronic hypoxia (14%  $O_2$ ) and sampled on day 15 and 19 of incubation. No differences in  $B_{max}$  were found between treatments on day 15, but on day 19 hearts from hypoxic embryos had lower  $\beta$ -adrenoreceptor density than normoxic embryos ( $5.0 \pm 2.1$  vs.  $8.1 \pm 2.7$  fmol  $\mu g$  protein $^{-1}$ ). Hypoxia did not modify receptor affinity at any developmental stage but affinity was lowered on day 19 as seen by the increased  $K_d$  values ( $0.07 \pm 0.02$  vs.  $0.19 \pm 0.06$  in normoxic 15d and 19d embryos respectively). This suggests that  $\beta$ -adrenoreceptor density is not affected by hypoxia until late gestation in broiler chickens and that receptor affinity is not affected by hypoxia, but is lowered with incubation time.

Keywords: Catecholamines, Beta-adrenoreceptor density, Broiler chickens, Developmental Physiology, Hypoxia

### A11.57 The Cyanobacteria *Nodularia spumigena*, the hepatotoxin nodularin and the Blue Mussel, *Mytilus edulis*

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Microcystins (MC) and nodularins (NOD) are cyanobacterial hepatotoxic peptides {Chorus and Bartram 1999 #11938}. The blue mussel, *Mytilus edulis* has been shown to accumulate nodularin at times of *Nodularia spumigena* blooms {Lehtonen, Kankaanpaa, et al. 2003 #9313} {Sipia, Kankaanpaa, et al. 2001 #6102}. The high stability of the toxin entering the food chain, can lead to the possible poisonings of humans, the loss of productivity for shellfish producers and adverse ecological impacts. Therefore, there is a need for accurate and sensitive assays to quantify this toxin in water and tissue samples.

A nodularin-based indirect competitive ELISA has been developed to increase the sensitivity of the assay with a lower minimum detection limit for nodularin in mussel tissue as compared to the microcystin-based protocol. It also yields a direct quantification of nodularin, compared to the commercial kits where it has to be expressed as MC equivalents. However, as in the MC-based assay, increasing salinity can give false-positives. This problem is easily overcome by using an appropriate saline control.

Keywords: Nodularin, Mytilus, Mussel, Nodularia, ELISA

### A11.58 Bioaccumulation of metals by *Mugil saliens* under chronic exposure to contaminated sediments: Gill histopathological changes

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The Esmoriz/Paramos coastal lagoon, is a habitat of ecological importance due to the presence of unique animal and plant species.

During the past few years its immersed area has been gradually reduced due to effluent discharges and siltation. The major contaminants in the lagoon are derived from a multitude of non-point sources associated with urbanization and industrial activities within the watershed. Heavy metals are probably a class of pollutants contributing to the observed decline of fish species. The metal content in the water and sediments in the lagoon were investigated as well as gill metal bioaccumulation and gill histopathological changes of the grey leaping mullet, *Mugil saliens*.

The extent of total metal contamination in the water was low, the metals in the particulate matter being the largest contribution. The estimated mean concentrations of each metal, in the sediment, that fish in the lagoon may have been exposed to, were 234 mg Zn/kg d.w. and 84 mg Cu/kg d.w. These values are below sediment quality guidelines.

The gills are the first organ to contact the contaminated sediment particles, so that they became a significant accumulation tissue as seen by the metal-bioaccumulation factors (BFA), Zn-BFA, 49% and Cu-BFA, 10%. The fish captured, with 6 to 12 years old, showed a significant increase in Cu- and Zn- (BFA), with fish age. Gill histopathological changes observed in epithelial tissue included edema, lifting of lamellar epithelium, severe vasodilatation, aneurisms, proliferation of filamentar epithelium and lamellar fusion suggesting chronic effects of metal exposure.

Keywords: Chronic exposure, *Mugil saliens*, Gill histopathology, Copper, Zinc

### A11.59 Gill lamella hyperplasia as a ecotoxicological indicator in Caspian Sea fishes

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The fish gill is a multi task organ that carries out the functions such as respiration, ion regulation, acid–base balancing and disposing nitrogenous by products. With area of more than 50% of the whole fish body, the fish gill is the most important part that affects by the toxins inside the water since it has very little immunity except of the operculum. This organ responds rapidly to various stimuli and is prone to chemical and physical damages. Therefore, when the gills expose to environmental pollutants for a long time they will experience various damages and complications. These tissue complications made the fish susceptible to secondary diseases and potential mortalities.

In this series of studies the gill tissue abnormalities had been studied in three important Caspian Sea fishes including *Acipenser Stellatus Acipenser Percicus*, and *Rtilus Frisi Kutum*. The gill specimens were H and E stained after fixation and preparing the tissue section in common methods.

Hyperplasia is the most common feature in all three species. In fact, these conditions are general response against pollutants, parasites, suspending particles or Ammonia or any other stimulus for adaptation or protection. Hyperplasia is an unusual multiplication of gill epithelium cells affecting the gas exchange and respiration and in more intense cases could merge the neighboring blades and hamper the gas exchange. According to our studies and some other researches, gill hyperplasia could be considered as an ecotoxicological indicator for fishes.

Keywords: Gill, Hyperplasia, Caspian Sea, Pollution