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C8 - General Thermal Biology

C8.1

Thermoregulating flowers: precision and limits of the biochemical regulatory mechanism

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Flowers of several families of seed plants warm themselves when they bloom. In some species, thermogenesis is regulated, increasing the rate of respiration at lower ambient temperature (T_a) to maintain a somewhat stable floral temperature (T_f). The precision of this regulation is usually measured by plotting T_f over T_a. However, such measurements are influenced by environmental conditions, including wind speed, humidity, radiation, etc. This study eliminates environmental effects by experimentally 'clamping' T_f at constant, selected levels and then measuring stabilized respiration rate. Regulating flowers show decreasing respiration with rising T_f ($Q_{10} < 1$). Q_{10} therefore becomes a measure of the biochemical 'precision' of temperature regulation: lower Q_{10} values indicate greater sensitivity of respiration to T_f and a narrower range of regulated temperatures. At the lower end of the regulated range, respiration is maximal, and further decreases in floral temperature cause heat production to diminish. Below this 'switching temperature', heat loss always exceeds heat production, so thermoregulation becomes impossible. This study compares three species of thermoregulatory flowers with distinct values of precision and switching temperature. Precision is highest in *Nelumbo nucifera*, (Q_{10} = 0.16) moderate in *Symplocarpus renifolius* (Q_{10} = 0.48) and low in *Dracunculus vulgaris* (Q_{10} = 0.74). Switching temperatures are approximately 30, 15 and 20 ^{o}C, respectively. High precision reveals a powerful inhibitory mechanism that overwhelms the tendency of temperature to increase respiration. Variability in the shape and position of the respiration-temperature curves must be considered in any explanation of the control of respiration in thermoregulatory flowers.

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C8.2

Effects of steady state temperature acclimation on cardiac myocytes in Pacific bluefin tuna *Thunnus orientalis*

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Bluefin tunas are apex predators renowned for their endothermic physiology, exceptional locomotory capabilities, and ability to inhabit a wide thermal range. Energy demand for the high performance life style of a tuna is sustained by elevated metabolic rates coupled with a unique enhanced cardiac performance. However, little is known about the molecular processes underpinning the phenotypic plasticity of tuna hearts across a temperature gradient. In this study we used a bluefin tuna specific microarray (8826 genes) and quantitative PCR techniques to explore the molecular responses to temperature (14^{o} C, 20^{o} C and 25^{o} C) acclimation in atrial and ventricular tissues of pacific bluefin tuna (PBFT). QPCR studies on genes involved in Ca^{2+} induced Ca^{2+} release pathway and global gene expression data analysis revealed that PBFT hearts have the capacity to alter their physiological process at cold (14) and warm (25) temperatures. Transcriptomic data indicated in changes in genes associated with energetic metabolism, ion transport, protein bio synthesis and cellular stress response showed significant changes in expression. A principal component analysis reveals that thermal response is tissue specific, with gene expression in atrium at 25^{o} C showing the greatest difference. In the ventricle, compared to spongy layer, compact layer appears to be more thermal labile. This study demonstrates that tuna hearts can optimize its performance by acclimating to 14^{o} C, potentially improving aerobic performance in the cold. Conversely the cardiac transcriptome at 25^{o} C is indicative of limitations in cardiac performance at warmer temperatures.

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C8.3

Getting to the heart(s) of cuttlefish: thermal sensitivity and mitochondrial function of cuttlefish hearts

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Cephalopod molluscs are exceptional athletic invertebrates that have evolved a high performance cardio-branchial system comprised of three hearts. As cellular powerhouses, mitochondria have central roles in aerobic heart function. This study tested the acute and chronic impacts of thermal challenges on acclimation and evolution of mitochondria from branchial and systemic hearts. Specimens from temperate and subtropical populations of the common cuttlefish *Sepia officinalis* were acclimated to 11°C, 16°C and 21°C. Permeabilised heart fibres were then used to assess mitochondrial function using high-resolution respirometry, and a substrate-inhibitor protocol; followed by measurements of mitochondrial content and glycolytic enzyme activity. Mitochondria showed varying temperature dependent fuel preferences with proline being a favoured substrate at high temperatures and carbohydrates at low temperatures. In addition, apparent contributions of the electron transport system relative to the phosphorylation system diminished with rising temperatures in temperate cuttlefish. This indicates very efficient mitochondrial coupling at thermal limits, where the effects of thermal acclimation of mitochondrial function were most evident. Smaller hearts with higher oxidative capacities were apparent in the subtropical specimens, while larger hearts with lower respiratory capacities were favoured in temperate specimens. Apparent fuel preferences for amino acids and carbohydrates as well as glycolytic enzyme activities also differed substantially between populations. In conclusion, this study provides evidence that *Sepia officinalis* hearts exhibit various bioenergetic adaptations in response to acute, seasonal, and evolutionary exposures to different habitat temperatures. This underlines the high adaptive plasticity of the common cuttlefish to cope with a broad range of thermal challenges.

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C8.4

Thermal tolerance of the first larval stage in Sub Antarctic crustaceans

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Thermal tolerance of early life history stages can influence the patterns of larval dispersal and thus species distribution. A mechanistic understanding of the physiological responses that affect larval survival is crucial to understand ecological patterns. Therefore, we investigated how thermal tolerance might contribute to setting distribution limits of crab larvae. The thermal tolerance windows for zoea I of *Lithodes santolla* and *Pagurus comptus*, two sub-Antarctic crustacean species, were determined. The thermal tolerance windows for zoea I were specified by lower and upper pejus (Tp) und critical (Tc) temperatures. The implications of our findings for the observed distribution of the two sub-Antarctic Crustacean species will be discussed, also considering the role of early life stages in defining the absence of reptant decapod crustaceans in the Antarctic.

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C8.5

Temperature dependent response to hypoxia involves haemocyanin functioning in the signal crayfish *Pacifastacus leniusculus.*

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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_2 demand, preserving internal PO_2 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 role of temperature in influencing the Hc O_2-affinity was examined for Hc from crayfish acclimated to 5°C, 13°C or 20°C. Increasing the temperature decreased the O_2-affinity of Hc, regardless of acclimation history of the crayfish. The response of Hc O_2-affinity to variations in L-lactate, urate, calcium and magnesium concentrations in the haemolymph were quantified as probable regulators of O_2-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_2-binding by Hc, but urate, Ca and Mg all potentiated the O_2-affinity although this was temperature dependent

for Ca. The *in vivo* functioning of Hc was assessed by determining the arterial and venous content and partial pressures of O_2 and CO_2, along with pH of haemolymph from crayfish exposed to progressive hypoxia of 1.2 kPa for 24hrs. Crayfish thermally acclimated to 5°C or 13°C exhibited a tolerance to sustained hypoxia for 24hrs, however this was not demonstrated in crayfish acclimated to 20°C, with 100% mortality after 24hrs.

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C8.6

Cold tolerance of the sub-Antarctic stone crab *Paralomis granulosa* is not limited by oxygen supply or high extracellular magnesium, but by uncompensated tissue functional capacity

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A low capacity for Mg^{2+} excretion has been proposed to constrain marine decapod crustaceans to waters warmer than 0°C. Furthermore, a study in a warm temperate crustacean has provided evidence for the concept of oxygen and capacity limited thermal tolerance. Here, thermal tolerance was determined in the sub-Antarctic crab *Paralomis granulosa* using an acute stepwise temperature protocol (-1, 1, 4, 7, 10, 13°C). Arterial and venous haemolymph oxygen partial pressure ($HLPO_2$), heart rate, ventilation rate and haemolymph cation compositon were measured at rest and after a forced activity (righting) trial. Haemolymph [Mg^{2+}] was experimentally reduced from 30 mmol L^{-1} to 10 mmol L^{-1} to investigate whether the animals get more active and tolerant to cold (-1, 1, 4°C). In controls activity was significantly reduced at -1 and 13°C compared to acclimation temperature (4°C). In contrast to findings in the warm temperate crab, arterial and venous $HLPO_2$ increased upon cooling even though heart and ventilation rate decreased. At rest cation composition was not affected by temperature change. After activity haemolymph [K^+] rose significantly at -1 and 1°C. Incubation at low [Mg^{2+}] did not result in an increase in activity and cold tolerance. In conclusion, oxygen delivery was not limiting in the cold. Mg^{2+} does not seem to play a role in cold tolerance of adult *P. granulosa*. Cold limitation in this subpolar species relates to the capacity limitation of tissue function, which does not benefit from the observed excess in oxygen availability.

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C8.7

Oxygen versus capacity and the time limitation of thermal tolerance: a conceptual analysis

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Climate warming emphasizes the need for a common understanding of thermal limitation by physiologists and ecologists. The whole organism responses to warming or cooling link to ecosystem response (Science 322, 690, 2008) and build on a suite of tissue, cellular, molecular and genomic events, in a systemic to molecular hierarchy of limitation (CBP 132A, 739, 2002). All of these are involved in setting limits to tolerance, shaping a species-specific, limited budget of tolerance over time beyond pejus limits. The limiting mechanisms are also the targets of processes shaping acclimatisation and evolutionary adaptation. The concept of oxygen and capacity limitation of thermal tolerance (OCLT) was proposed as a matrix integrating the levels of biological organisation and the synergistic effects of environmental stressors (JEB 213, 881, 2010). Recent work has emphasized its role in heat tolerance. Here, capacity and oxygen limitation go hand in hand. A mismatch in oxygen supply versus demand causes a limitation in aerobic scope and finally transition to anaerobic metabolism, paralleled by the development of molecular stress events. The respective picture is less clear during cold exposure, partly related to the climate regime. In fact, functional characters in polar species may reflect adaptation to excess oxygen availability rather than limitation (Deep Sea Res. II 53, 1071, 2006). Reduced metabolic rates and excess ambient oxygen will alleviate oxygen limitation in the cold such that cold limitation by an overall loss in functional capacity is more stringent than by reduced oxygen supply.

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C8.8

Physiological responses to thermal ramping in three life stages of a Tenebrionid beetle

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Understanding the physiological limitations of temperature tolerance is an important step in characterizing how organisms will respond to significant changes in their thermal environments. Upper thermal limits are typically assessed in animals by ramping temperature

at a set rate until a marked decline in physiological performance occurs. Despite the widespread use of this technique for assessing thermal tolerance, the physiological responses to thermal ramping are not well understood. We assessed thermal tolerance in three life stages (larvae, pupae, and adults) of Tenebrionid beetles (*Tenebrio molitor*) by measuring CO_2 release during thermal ramping (thermolimit respirometry). We recorded three independent estimates of the critical thermal maximum (CT_{max}) for each animal: 1) temperature at peak CO_2 release, 2) temperature at the last breath, and 3) temperature before a final CO_2 burst. We compared these CT_{max} estimates among the three life stages, each of which produced a unique pattern of CO_2 release. All three measures of CT_{max} were statistically identical among the three life stages. These results demonstrate that CT_{max} does not vary significantly among different life stages of this species, despite widely varying environments and behavior. Additionally, our results suggest that each of the three CT_{max} estimates we used may be reliable measures of thermal tolerance.

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C8.9

Latitudinal variations in the metabolic rates of amphipods: a large-scale perspective.

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To determine if there is a relationship between rates of energy consumption and biogeography in aquatic ectotherms, we carried out a meta-analysis of the standard metabolic rates of 48 species of amphipod crustaceans that occur along a broad thermo-latitudinal gradient (60˚S-79˚N, 0-25˚C). When phylogeny and differences in methodology (e.g. closed or flow-through respirometry, holding time), nutritional status and ecotype were accounted for, metabolic rates showed a significant decrease with latitude suggesting the absence of metabolic cold adaptation (MCA) in amphipods. The thermal sensitivity of metabolic rate, calculated across species (Q_{10} = 1.99) was very close to that predicted from a temperature-dependent rate function, further suggesting a lack of thermal compensation. When the effect of temperature was removed, the effect of latitude on metabolic rate was weaker but still significant, indicating that temperature is the primary but not the only latitudinal variable associated with low metabolic rates in polar amphipods. We suggest that environmental stability, seasonal food availability, and phylogenetic history may also favour the selection of low costs of living at high latitudes.

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C8.10

The cold tolerance of successful global invader: acute response and extended acclimation.

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The successful colonization of a new area is principally based on species capability to cope with the novel environment and efficiently acclimatize. Several marine ectotherms are high successful global invaders, among them *Carcinus aestuarii* and *C. maenas*. These species have established populations in five different temperate regions outside their native range in Europe, constituting one of the 100 most invasive species (IUCN). With the aim to evaluate the adaptive success and forecast the spread of introduced populations we analyzed their tolerance window at both level of acute responses and extended acclimation. We carried out a latitudinal comparison between populations of *C. aestuarii* and *C. maenas* to test the oxygen and capacity limitation of cold tolerance as well as to estimate the change in metabolic and activity regimes during acclimation. The evaluation of cardiac effort together with the venous and arterial pO_2 revealed a sudden decrease of metabolic performances and activity when animals move from temperate (10°C) to colder (2°C) water. The metabolic quiescence occurring during the acute exposure is followed by an efficient acclimation to the new environmental conditions. The appraisal of activity patterns demonstrated prompt response of animals acclimated at 5°C acclimated, while delayed at 2°C. Thus the two *Carcinus* species show an initial capacity limitation, followed by a progressive recovery and efficient acclimation to the new thermal regimes. The pronounced phenotypic plasticity likely contributes to the successful invasion of *Carcinus* and allows us to forecast a wider spread of these species in areas colder than the natives.

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Poster Session 17:00 – 19:00 Thursday 1st July 2010

C8.11

Thermal influence on fitness related life-history traits in a tropical gecko *Paroedura picta*

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In ectotherms, environmental temperature is the most prominent abiotic factor modulating life-history traits. Tropical ectotherms have narrow thermal niches and thus live near temperature enabling maximum performance. After slight temperature increase above this threshold, their performance and hence fitness is expected to be lower. We report results of an experiment on effect of three constant temperatures on body size and reproductive rate in the Madagascar ground gecko (*Paroedura picta*). Freshly-laid eggs were randomly distributed into three climatic chambers differing in constant temperatures (24, 27, 30 °C). After hatching, each individual was raised separately in its respective climatic chamber till reaching final body size. The influence of ambient temperature on final body size was different in respect with sex. Final body-size in males was the largest at the intermediate temperature, which could bring advantage in intrasexual selection via success in male-male combats. Final body size seems to be more canalized in reproductive females, where only the lowest temperature led to significantly lower size. However, pattern in either sex does not conform to otherwise widely observed temperature-size rule in ectotherms (larger final body size at lower ambient temperatures). Increasing temperature positively influences female reproductive rate, but in a manner not predicted by a simple model of reaction kinetics (Boltzmann-Arrhenius principle). The results in *P. picta* suggest that prediction of fitness consequences of shifts in thermal environment can be complicated by different magnitudes and even directions in changes in different fitness-related traits across sexes

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C8.12

Effects of incubation temperature on proliferation and myogenic differentiation of dermomyotome cells and their consequences on muscle growth in fish

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Muscle growth in teleost fish is a plastic process that is strongly influenced by external factors. Perhaps the most important among these factors is temperature. Related effects are heterogeneous and include the phenomenon that the thermal experience during embryonic life is likely to become 'imprinted' and to have a lasting influence on muscle cellularity and growth later in ontogeny. However, the causes for this phenomenon at the precursor cell level are as yet unknown. The here presented data are part of a more detailed long-term study using digital morphometry and immunodetection of molecular markers (Pax7, MEF2, H3P) to investigate temperature-related changes in muscle growth dynamics in relation to myogenic precursor cell proliferation and differentiation rates in brown trout. They describe the situation in the late phase of the imprinting period before fish with different early thermal experience are transferred to similar (ambient) temperature conditions at hatching. A specific focus is set on the dermomyotome, which is known to be the main (and possibly exclusive) source of the myogenic precursors that subsequently account for myotome expansion. Preliminary results suggest that differences between thermal groups at hatching are stem cell-based rather than cellularity-based.

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C8.13

Synergistic impacts of ocean acidification and temperature increase on the physiology of *Mytilus edulis*

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Ocean acidification induced by anthropogenic CO_2 is expected to cause a progressive reduction in surface ocean pH reaching almost 0.8 pH units over the next 300 years (IPCC 2007) and affecting marine species and ecosystems. Cellular processes strongly depend on pH, therefore, acid-base regulation is crucial for the performance of marine organisms, however, at the expense of high energetic costs (Pörtner, 2008). Furthermore, increasing CO_2 concentrations may lower the capacity for oxygen supply in invertebrates. Together with rising temperatures, small pH changes might therefore already exert critical impact on physiological processes in marine organisms.
We have studied the synergistic effects of increasing temperature and CO_2 levels on energy metabolism, thermal tolerance and acid-base regulation capacity of the blue mussel *Mytilus edulis*. Long-term exposures at intermediate temperature did not show critical CO_2 impacts under moderately elevated CO_2 levels (≤ 1120ppm for 90d). A slight decrease in acid-base status followed the alphastat pattern. At 3000ppm pH fell initially, was stabilised by HCO_3^--accumulation and remained uncompensated during the incubation period. During acute warming (10 to 31°C; 3°C/night) CO_2-exposed mussels (750, 1120ppm) showed a stronger increase in respiration rate leading to higher Q_{10}-values than seen in controls. Accordingly, CO_2-exposed mussels

showed a stronger temperature-dependent increase in heart rate. Anaerobic metabolites accumulated above 28°C defining the upper critical temperature which was independent of the CO_2 -levels applied. We hypothesize that CO_2 exposure may affect pejus more than critical limits and causes a narrowing of the thermal window of the species.

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