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C5–GENERAL THERMOBIOLOGY

C5.1

What determines a species' geographic range? Thermal biology and geographical range size relationships in European diving beetles

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Most species are rare, few are common? Understanding the factors determining the geographic range size of organisms is a key unresolved issue in ecology. This is crucial if we are to understand how organisms may respond to global climate change. In Europe some taxa have been successful postglacial northward migrants, whilst others have remained 'locked' in their southern refugia. What makes a good postglacial colonist, and why are some species more widespread than their relatives? Widespread species are generally assumed to have broader fundamental niches than narrow range taxa, but there is limited evidence for this.

We attempted to correlate a number of physiological traits, namely upper and lower thermal limits, thermal range, acclimatory ability of species and inter-individual variability of these traits with the size and latitudinal position of their geographical range. Three taxonomically defined assemblages of diving beetles (*Deronectes* spp., *Ilybius* spp. and *Agabus* spp.) were chosen as ideal model systems for the investigation of factors controlling range size. All three groups combine closely-related widespread species and narrow-range Mediterranean endemics, which have similar general ecologies. Here we provide a formal test for the 'niche-breadth hypothesis' and the 'climatic variability' hypothesis within a phylogenetic framework.

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C5.2

Thermal niche, aerobic scope and temperature sensitivity: A latitudinal comparison

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Most ectotherms have optimal speeds for activity at relatively high body temperatures, close to their upper limits; however, comparative studies are often hampered by phylogenetic differences among study species. A study of burrowing capacities within the bivalve genus *Laternula* was conducted on the Antarctic *L. elliptica* (67°S), Australian *L. gracilis* (38°S) and Singaporean *L. truncata* (1°N) to test the prediction of symmorphosis, that organisms will evolve an aerobic scope sufficient to complete their life cycle under their experienced thermal regime. Both Antarctic *L. elliptica* and tropical *L. truncata* live in stable thermal environments (3 and 4 °C annual range respectively) and their upper temperature limits of activity, the temperature where 50% of animals lose their ability to re-bury, are only +1 and +3 °C degrees above the annual mean maximum temperature (1 and 31 °C) respectively, they are stenothermal. *L. gracilis* live in an environment where sea-water temperatures range from 10 to 22 °C (Port Phillip Bay, Australia) and have an upper temperature limit for burying of 32 °C; +10 °C above the temperature maxima, they are eurythermal. The optimum temperature for burrowing in all species was closely correlated with maximum environmental temperature. At their mean annual temperature *Laternula* are the slowest recorded burrowing bivalves, but *L. elliptica* had a higher burrowing speed than would be expected due to environmental temperature alone. This was not due to elevated mitochondrial density, as is

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seen in Antarctic fish, but could be due to morphological adaptation of the foot.

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C5.3

Thermal tolerance in the lugworm *Arenicola marina*: Measures of climate dependent organismal performance

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The lugworm *Arenicola marina* is distributed over a wide latitudinal range and exposed to highly fluctuating temperature conditions within seasonal as well as tidal cycles. Animals are specialized to adjust to ambient climate conditions as well as climate variability as evidenced from patterns of latitudinal adaptation and seasonal acclimatisation of thermal tolerance windows. These and associated temperature dependent performance optima were elaborated from in vivo measurements of protein synthesis in artificial burrows and from the quantification of digging activity in natural sediments. The respective results in fact revealed differences in location, width and height of performance curves on the temperature scale between populations from the French Atlantic coast, the German North Sea and the Russian White Sea in accordance with temperature dependent latitudinal adaptation and seasonal acclimatisation. Quantification of the organismal performance range is thus relevant in the light of global warming and climate change, because long-term exposure to temperatures beyond the respective performance window leads to, for example, restrictions of digging activity with the consequence of extended exposure to predators or restrictions of growth. Both phenomena would result in decreased local abundances and eventually in a shift in geographical distribution. This study is part of a joined research project with Münster University (Animal Physiology) within the DFG priority programme AQUASHIFT.

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C5.4

Thermal tolerance in the lugworm *Arenicola marina*: Burrow irrigation and respiration

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The lugworm *Arenicola marina* is a model organism from the intertidal zone. It is widely distributed, very abundant and shapes the ecosystem by bioturbation. As the worm spends nearly all of its life within the sediment, it is forced to pump oxygenated water through the burrow for respiration. We used artificial burrows to mimic natural conditions and investigated animals of different seasons originating from the North Sea coast with respect to their ventilatory activity and respiration rates during

exposure to different temperatures. Within a thermal window weight specific volume flow and oxygen consumption rose exponentially according to the Q_{10} relationship. Beyond certain temperatures a deviation from the temperature–velocity relationship was observed. We propose that these temperatures are characterized by the onset of anaerobic metabolism and correspond to the critical temperatures. Evaluation of burrow irrigation patterns and oxygen extraction depending on temperature furthermore shows thermal optimum ranges beyond which survival is not at immediate risk, but performance is already reduced. Comparisons of thermal tolerance windows in animals from various seasons support an analysis of potential consequences of climate change on the well-being and geographical distribution of the species. This study is part of a joined research project with Münster University (Animal Physiology) within the DFG priority programme AQUASHIFT.

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C5.5

Oxygen dependence of post- CT_{max} CO_2 release in *Drosophila*

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Thermolimit respirometry is utilized to investigate aerobic metabolism at temperatures up to and beyond the upper critical thermal limit (CT_{max}) in tracheate arthropods. CT_{max} has been shown to be negatively affected by hypoxia in accordance with Pörtner's oxygen limitation hypothesis. Unexpectedly it was found that CO_2 emission, having initially decreased after CT_{max} , proceeded a few minutes later to peak several fold above previously maximal levels. It was speculated that the cause of this large increase in CO_2 flux might be discharge of internal bicarbonate by acidification, or – more interestingly – mitochondrial respiration. The aim of this study was to determine if the high post- CT_{max} CO_2 flux was coupled to aerobic processes.

In a series of experiments the CO_2 flux of individual *Drosophila* was monitored while temperature was ramped upward. At CT_{max} CO_2 flux decreased and animal activity ceased. The gas flowing through the experimental chamber was then switched from air to nitrogen to establish anoxic conditions. Our null hypothesis was that anoxia would not affect the post-mortal CO_2 flux.

The result of anoxia was an immediate exponential decay of CO_2 flux and total extirpation of the post- CT_{max} CO_2 peak. We therefore disproved our null hypothesis and demonstrated that the post- CT_{max} CO_2 peak was aerobic. Because the amplitude of the post- CT_{max} CO_2 peak was several fold above maximal CO_2 flux levels attained by the viable organism (presumably caused by mitochondrial uncoupling), the sensitivity of CT_{max} to hypoxia is probably not caused by limitations in tracheal gas exchange.

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C5.6**Regulation of mitochondrial functioning with temperature in ectothermic animals**

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Alterations in mitochondrial functions are proposed as key processes in seasonal as well as evolutionary temperature adaptations of ectotherms. Cold exposure frequently induces mitochondrial proliferation to cope with the decelerating effects of low temperatures. In populations of cod (*Gadus morhua*) along a latitudinal cline differences at the functional level became evident mostly at cold temperature with higher mitochondrial capacities in the sub-Arctic population. In metabolically active tissues like liver, cold acclimation was found to comprise a shift between mitochondrial functions, indicated by increased activity ratios of the mitochondrial key-enzymes citrate synthase (CS) and cytochrome-*c* oxidase (COX). In all cases transcript levels and functional capacities were not tightly correlated with enzyme activities during thermal adaptation and thereafter, though transcriptional processes are clearly involved and seem to be important to establish the new steady state. However, cold exposure of isolated hepatocytes prepared from the common eelpout (*Zoarces viviparus*) resulted in increments in CS as well as COX capacities contrasting the situation in whole animal acclimation. This suggests that systemic signals coordinate the pattern of adaptation at the organismic level. As adenosine levels rose transiently during cold acclimation of eelpout in vivo, we tested its role as a regulatory signal during thermal acclimation. In isolated hepatocytes, adenosine had in fact a suppressing effect on COX activity and total RNA contents, but most striking was the activation of COX expression, found for both, mitochondrial and nuclear encoded subunits. Finally, a pattern of expression results which is similar to the one observed in vivo.

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C5.7**The presence and function of uncoupling proteins in a phylogenetically ancient mammal, the rock elephant shrew, *Elephantulus myurus***

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The success of early mammals is often attributed to the ability to produce heat endogenously through non-shivering thermogenesis (NST). In eutherian mammals NST is known to occur in brown adipose tissue (BAT) where uncoupling proteins (UCPs), particularly UCP1, work to generate heat instead of ATP. Until recently, UCP1 was thought to be exclusively associated with NST in eutherian mammals. A recent study, however, demonstrated that UCP1 appears in the vertebrate lineage as early as

before the divergence of ray-finned and lobe-finned fishes although a thermogenic function of UCPs has not been shown in fishes. This begs the question of where in the vertebrate lineage UCP1 gained a thermogenic function. The Afrotheria are at the base of the eutherian radiation and present the most logical place to investigate a thermogenic role of UCPs. The rock elephant shrew, *Elephantulus myurus* (Macroscelidea: Afrotheria) shows a thermogenic response to noradrenalin (NA). However, at present it is unclear whether this thermogenesis is mediated by UCP1 and recruited in the cold. The aim of this study is therefore to investigate the occurrence and function of uncoupling proteins in *E. myurus*, under cold and warm acclimation. In vivo measurements revealed no difference in the NA induced NST capacity of cold and warm acclimated animals. Using Western blotting, we detected UCP1 and UCP3 in BAT and skeletal muscle, respectively. Our current experiments are investigating whether cold acclimation elevates UCP mRNA levels as well as the thermogenic function of UCP1 through proton leak kinetics of BAT mitochondria.

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C5.8**Antarctic sea ice: Habitat characteristics, metazoan fauna, and adaptations to low temperature**

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The sea ice of polar regions represents one of the largest ecosystems on earth with specially adapted organisms. In the Southern Ocean alone, up to 20 million km² are covered by sea ice in winter. Sea ice is not a solid block, but permeated by small brine channels which house a diverse fauna and flora. With an average temperature below $-1.9\text{ }^{\circ}\text{C}$ and temperature extremes of down to $-22\text{ }^{\circ}\text{C}$, these channels represent one of the coldest environments on earth. Coupled to the temperature, the brine salinity can vary between $S=220$ at $-22\text{ }^{\circ}\text{C}$ and $S=2-3$ near $0\text{ }^{\circ}\text{C}$ ice temperature. Surface, interior, bottom and sub-ice habitats characterised by different environmental factors are associated with sea ice and play different roles in the life cycles of sympagic (ice-associated) species. Several copepod and turbellarian species are the dominant members of the Antarctic sympagic meiofauna. Others, recently in pack ice identified species are the ctenophore *Callianira antarctica* and the nudibranch *Tergipes antarctica*. In-situ studies show that harpacticoid copepods and turbellarians cope well with temperatures down to $-4\text{ }^{\circ}\text{C}$ and corresponding salinities of 69. The calanoid copepod *Stephos longipes* is well adapted to a temperature range of $-1.2\text{ }^{\circ}\text{C}$ ($S=25$) and $-3.1\text{ }^{\circ}\text{C}$ ($S=55$). This species is slightly hyperosmotic to seawater in a range of

$S=25$ to 45 and exhibits a thermal hysteresis of 2° ($+1^\circ$). Analysis of a cDNA-library of *S. longipes* enriched for genes expressed at -3.1°C revealed the presence of a putative antifreeze protein.

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C5.9

Adaptation of ion regulatory capacities in gills of cold and warm water fish under hypercapnic acidosis

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The currently rising CO_2 levels in the atmosphere and in marine surface waters combine with trends of increasing temperatures. Simultaneous shifts in those factors will likely enhance sensitivity to environmental extremes especially in stenothermal animals. The preservation of ion balance and the regulation of pH despite environmental fluctuations play a special role in this context, and support the maintenance of vital cellular functions but also comprise a significant fraction of the energy budget. Firstly, we investigated the time course of acclimation to hypercapnia (10,000 ppm) in the benthic eelpout *Zoarces viviparus* at optimum temperature. Whole animal oxygen consumption was not affected by acute hypercapnia. However, the expression of several ion transporters in gills changed during the acclimation process, indicating different responses at the mRNA levels over time. Whereas passive transporters were reduced during the early phase of adaptation and recovered towards the end, Na^+/K^+ ATPase expression remained high and doubled until the end of the experiment. Most strikingly, $\text{Na}^+/\text{HCO}_3^-$ cotransporter expression, which initially displayed a similar pattern as the other passive transporters, was increased about threefold towards the end. The functional significance of these changes at cellular and tissue levels is under current investigation and will be compared with the respective patterns in cold adapted Antarctic fish.

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C5.10

Regeneration rates in the Antarctic Brittlestar: *Ophionotus victoriae*

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The most familiar feature of echinoderm physiology is their remarkable capacity for limb regeneration. This is an entirely natural phenomenon underlying a sacrificial defence mechanism. However, there is clearly an energy balance between

organism survival and the cost of producing a new limb. The question is does such a cost impinge on the ability of the echinoderm to adapt to changing environmental conditions, such as increased seawater temperatures as a result of climate change? Development is slow in Antarctic marine ectotherms compared to temperate and tropical species and a number of Antarctic invertebrates have been proven to be particularly sensitive to increases in environmental temperatures. With very few exceptions, they have survival temperature envelopes between 5°C and 12°C above the minimum sea temperature of -2°C . However essential biological activities often cease well below these thresholds. In these cases, behavioural changes have been studied as a metric of capacity to cope with change. In this study we use the Antarctic brittle star, *Ophionotus victoriae*, a scavenger feeder, as a model to determine how limb regeneration costs affect survival in a changing environment. We present data on limb damage, regeneration rates and thermal sensitivity of *O. victoriae*, as part of our ongoing project into the thermal adaptation and biology of Antarctic invertebrates.

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C5.11

Differential expression of three *FoxK1* splice variants in relation to embryonic temperature in the tiger pufferfish

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FoxK1 is a member of a highly conserved family of transcription factors that are known to play key roles in various cellular processes, including development and oncogenesis. We found that the tiger pufferfish (*Takifugu rubripes*) orthologue of mammalian FoxK1 has 7 exons, is located in a chromosomal segment of conserved synteny between pufferfish and mouse, and is expressed as three alternative transcripts, designated TFoxK1- α , TFoxK1- β and TFoxK1- γ . The isoform TFoxK1- α is the orthologue of mouse FoxK1- α and corresponds to a putative protein of 558 residues that contains the forkhead and forkhead-associated domains typical of Fox proteins and shares 53% global identity with its mammalian homologue. TFoxK1- β and TFoxK1- γ arise from intron retention events and contain premature stop codons, indicating that these transcripts are likely to be degraded by nonsense-mediated decay, an important mechanism that regulates transcript levels. TFoxK1 splice variants were differentially expressed with respect to embryonic stage and between fast and slow myotomal muscle in adult fish. The TFoxK1- α protein was expressed in myogenic progenitor cells of fast myotomal muscle. During embryonic development TFoxK1 was transiently expressed in the developing heart, brain, eye and somites. The ratios of the three alternative transcripts at equivalent developmental stages were different for embryos reared at 15, 18 and 21°C . Our data suggest that

TFoxK1 may play a role in the developmental plasticity of myogenesis in *T. rubripes* reared at different temperatures.

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C5.12

Reduced temperature tolerance in cadmium-exposed oysters: Early mismatch between O₂-demand and O₂-supply due to impaired aerobic capacities

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Marine ectotherms inhabiting coastal waters and estuaries such as oysters are exposed to variable environmental conditions. In light of global change, with increasing annual mean temperatures and temperature extremes, additional stressors like pollution might become of higher risk. Based on the concept of oxygen-limited thermal tolerance in aquatic ectotherms (Pörtner and Knust, Science 2007), we show that a persistent pollutant, cadmium, can have detrimental effects on oysters (*Crassostrea virginica*) by reducing thermal tolerance due to

impaired energy metabolism and elevated costs of basal maintenance. During acute temperature increase (4 °C/12 h) standard metabolic rate rose in both control and cadmium-exposed (50 µg Cd²⁺ L⁻¹) animals, with a consistently higher SMR in Cd-exposed oysters at all temperatures. In addition, compared to their control counterparts Cd-exposed oysters had lower hemolymph oxygen levels during temperature rise indicating that oxygen delivery systems cannot provide enough oxygen to cover elevated metabolic demands. Interestingly, heart rates and contraction amplitudes were similar in control and Cd-exposed oysters at all studied temperatures suggesting that cardiac output does not increase to compensate for elevated energy demand in Cd-exposed oysters. These observations suggest a limitation in oxygen uptake via ventilation rather than in oxygen distribution via circulation. Overall, exposure to Cd at high temperature results in functional hypoxia in oysters potentially limiting their aerobic scope and thus the temperature tolerance window.

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