3.

The role of the lactate dehydrogenase of *Daphnia magna* and *Daphnia pulex* for the tolerance of elevated temperatures

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Poikilothermic plankton organisms such as *Daphnia* have to cope with major fluctuations of the abiotic factors oxygen and temperature in their habitat. Limitation of temperature tolerance is related to a mismatch of oxygen supply and demand, which is indicated by the production of the anaerobic end product l-lactate. Comparing lactate accumulation in *Daphnia magna* and *Daphnia pulex*, major differences in their capacity to tolerate elevated temperatures by making use of anaerobic energy metabolism could be observed. While lactate formation in *D. magna* occurred at high temperatures and changed with acclimation, only low contributions of anaerobic metabolism were found in *D. pulex*.

Characterization of the lactate dehydrogenase (LDH) of both species revealed temperature-induced effects on enzymatic activity and affinity. Particularly, the inhibition by high pyruvate concentrations was differently influenced by temperature in both species. While substrate inhibition is diminished at enhanced temperature in *D. magna*, the enzyme activity is more strongly inhibited by high pyruvate concentrations in *D. pulex*. As a consequence, LDH activity cannot be increased by pyruvate accumulation under heat stress in this species. Thus the enzymatic properties of LDH, the key enzyme of anaerobic metabolism, could play a decisive role for the lower thermotolerance of *D. pulex*.

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4.

Field study of the molecular and metabolic responses of the bivalve *Modiolus barbatus*

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In light of global warming and taking into account its impact on life in several levels of biological organisation, the bivalve *Modiolus barbatus*, commonly known as bearded-horse mussel, was studied with regard to a number of biochemical and metabolic responses. This study aims to explore the thermal limits of the aforementioned bivalve within its natural habitat, using stress bioindicators, such as enzymes of the glycolytic catabolic pathway and some Heat-Shock Proteins (HSP).

Mature individuals were collected from Thermaikos Gulf and placed within three different thermal niches in the water column (3 m, 10 m, and 17 m). The mantle and posterior adductor muscle were sampled monthly and frozen in liquid nitrogen *in situ*, from November 2007 until August 2008. Furthermore, the samples were scanned for any evidence of field mortality. Maximal enzyme activity of the glycolytic enzymes hexokinase (HK), phosphofructokinase (PFK), aldolase (ALD) and pyruvate kinase (PK), as well as the expression of HSP90 and HSP70 were measured in the laboratory, so as to address potential diverse response concerning the depth and season.

Compared to 10 m and 17 m, mussels' mortality increased significantly in August at 3 m when surface sea-water temperature raised as high as 28 °C. These data indicate that bearded-horse mussel of this particular ecosystem (Thermaikos Gulf) are most likely approaching their upper thermal tolerance limits. Metabolic response was mainly raised by the increase of the PFK and PK activities. The seasonal pattern of the HSP expression was intense in 3 m rather than in 10 m in all cases, but hardly noticeable in 17 m.

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5.

Evidence for close relationship between anaerobic metabolism and Heat Shock Response (HSR) in the marine bivalve *Mytilus galloprovincialis*

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Mussels are naturally subjected to an environment of low oxygen availability, since during low tide they are exposed to the air for hours. Survival time during anoxia is strongly influenced by several factors such as temperature. Elevated temperature is known to induce the expression of heat shock proteins (Hsps) in mussels' tissues. In this study, we investigated thermal tolerance during hypoxia and prolonged periods of associated thermal stress in *Mytilus galloprovincialis*. Metabolic responses were analyzed by studying the activity of key glycolytic enzyme pyruvate kinase (PK). To determine when thermal stress is initiated in the tissues of exposed to air mussels, the expression levels of Hsp70 and Hsp90 were measured.

Adult mussels that had been acclimated at water temperatures of 18 °C (group A) and 26 °C (group B), were exposed at air temperatures of 18 °C or 32 °C (those from group A) and 32 °C (those from group B). Mussels from all groups were sampled at regular periods, mantle and posterior adductor muscle (PAM) were dissected, frozen and stored at −80 °C before used for the measurement of PK activity and Hsp70 and Hsp90 levels.

According to our results, exposed *M. galloprovincialis* cannot tolerate air temperatures as high as 32 °C for more than 20–25 h. Furthermore, the expression of both Hsps under all conditions of exposure indicates that, except of temperature, hypoxia by itself activates the HSR of mussels. Taking into account mussels' pattern of metabolic adaptations to long-term anoxia, it seems that intracellular acidocis triggers HSR in mussel tissues.

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6.

Ambient temperature during pregnancy entails short-term and long-term effects on cortisol levels in mother and offspring Guinea pigs

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Ambient temperature influences almost all biological processes from molecules to organisms and ultimately populations. We experimentally examined the influence of contrasted ambient temperature on pregnant and non-reproductive female guinea pigs (*Cavia aperea porcellus*), and on their offspring. This study notably focused on the impact of temperature on maternal body mass variations, stress assessed via hormone levels (glucocorticoid) and behavior. Twenty female guinea pigs were placed under warm 22 ± 1.5 °C thermal regime; twenty others were maintained under cold 15 ± 1.5 °C conditions. In each group, twelve females were pregnant and eight were non-reproductive. Surprisingly, thermal environment did not affect maternal mass or the quantity of food ingested. Such unexpected results suggested that physiological and/or behavioral regulations

associated with energy utilization occurred. Pregnant females exhibited higher basal plasma cortisol levels compared to non-reproductive females. Pregnant females maintained under cold conditions shown lower basal level of cortisol, but they also displayed greater amplitude of stress response than warm females. Females kept under warm conditions were more active compared to the cold females. Pregnancy entailed a chronic elevation of stress hormone likely associated with increased energy demands; such effect was down modulated by cold conditions with compensation via a lower activity level. Interestingly, the lower basal cortisol level and the greater stress response amplitude of the cold females were transmitted to their pups, suggesting that the hormonal ambience experienced during development shaped the stress post natal cortisol profile.

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POSTER PRESENTATIONS

7.
Temperature dependent regulation of cardiac excitability by sarcolemmal potassium currents in fish

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Sarcolemmal potassium currents are essential for excitability of the heart and thermal plasticity of cardiac function in ectothermic vertebrates. In fish cardiac myocytes, duration and repolarization of cardiac action potential (AP) are regulated by inward rectifier potassium currents (I_{K1}, $I_{K,ATP}$) and delayed rectifier potassium currents (I_{Kr} and I_{Ks}) which are modified by acute and/or chronic temperature changes. The rapid component of the delayed rectifier current (I_{Kr}) is almost ubiquitously up-regulated in cold-acclimation (+4°°C) of fish hearts via increased expression of the ERG channels. Accordingly, I_{Kr} suggested being "cold-adaptive" by preventing excessive prolongation of AP in the cold. In most fish species, also I_{K1} is up-regulated in the cold, with the exception of salmonid fish where the current is depressed. The I_{K1} current is mediated by three different inward rectifier channels, Kir2.1, Kir2.2 and Kir2.5 subtypes, and thermal response of the I_{Kr} seems to be dependent on the expressed isoform: Kir2.2 is the dominant form in warm-acclimated (+18 °C) fish, while the Kir2.5 is responsible for the cold-acclimation of the I_{K1}. Salmonid fish obviously do not express Kir2.5 which may explain the depression of their I_{K1} in the cold. $I_{K,ATP}$ is activated under acute heat stress, suggesting that ATP-sensitive potassium channels of the fish heart are cardioprotective under extreme thermal conditions. The I_{Ks} is not modified by thermal acclimation and it may function as repolarization reserve to secure cardiac excitability if other potassium currents fail.

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8.
Effect of temperature acclimation on sarcoplasmic reticulum calcium content in atrial and ventricular myocytes of the fish heart

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Ryanodine (Ry) sensitivity of cardiac contraction differs between teleost species, between atrium and ventricle, and according to the thermal history of the fish. The hypothesis that variability in Ry-sensitivity of contraction is due to temperature-related, species-specific and chamber-specific differences in the sarcoplasmic reticulum (SR) Ca^{2+} content, was tested by comparing steady-state (SS) and maximal (Max) Ca^{2+} loads of the SR in three teleost fish, rainbow trout (*Oncorhynchus mykiss*), burbot (*Lota lota*) and crucian carp (*Carassius carassius*), which differ in the extent of SR contribution to e–c coupling. Fish were acclimated at 4 °C (cold-acclimation, CA) or 18 °C (warm-acclimation, WA) and SR Ca^{2+} content was released by a rapid application of 10 mM caffeine to single cardiac myocytes and its amount was determined from the Na^{+}–Ca^{2+} exchange current at 18 °C. SS Ca^{2+} load was larger in atrial (304–915 μmol l^{-1}) than ventricular (224–540 μmol l^{-1}) myocytes in all fish species ($P<0.05$) and the same was true for Max SR Ca^{2+} content: 550–1522 μmol l^{-1} and 438–840 μmol l^{-1} for atrial and ventricular myocytes, respectively ($P<0.05$). Consistent with the hypothesis acclimation to cold increased Ca^{2+} load of the cardiac SR in the burbot heart, but contrary to the hypothesis, temperature acclimation did not affect SR Ca^{2+} content in rainbow trout and crucian carp hearts. Overall, an inverse relation between SR Ca^{2+} content and Ry-sensitivity of contraction force (and Ca^{2+} sensitivity of RyRs) seems to prevail in fish hearts.

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