

A10.29 PRE-ACCLIMATION TO LOW AMMONIA IMPROVES AMMONIA HANDLING IN COMMON CARP WHEN EXPOSED SUBSEQUENTLY TO HIGH ENVIRONMENTAL AMMONIA

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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We tested the hypothesis whether acclimation with low concentration of ammonia can facilitate the fish to tolerate subsequent sub-lethal ammonia exposure by activating ammonia excretory pathways. Common carp were pre-exposed to 0.27 mM ammonia (~10% 96 h LC₅₀) for 3, 7 and 14 days. Thereafter, each of these pre-exposed and parallel control (without pre-exposure) groups were exposed to 1.35 mM high environmental ammonia (HEA, ~50% 96 h LC₅₀) for 12 h and 48 h. Results show that ammonia excretion rate (J_{amm}) was strongly inhibited (or even reversed) in control group following HEA. On contrary, pre-acclimated fish (typically 3 and 7 days) were able to maintain J_{amm} at basal level. The efficient ammonia efflux in pre-acclimated fish was associated with the up-regulation of branchial mRNA expression of ammonia transporters and exchangers. Pre-acclimation stimulated the expression level of Rhcg-amRNA; significant up-regulation was recorded during HEA exposure in pre-acclimated group relative to the control-HEA exposed group. No positive effect of pre-acclimation was noted for Rhbg. Relative to control, the transcript level of Na⁺/H⁺ exchangers was remarkably elevated in ammonia pre-acclimated fish and remained higher during the subsequent HEA exposure. Similar trend was noted for mRNA expression of Na⁺/K⁺-ATPase, however, expression level of H⁺-ATPase remained unchanged in all the experimental conditions. In conclusion, our study clearly demonstrates that although the pre-exposure to a low dosage of ammonia did not induce that many measurable effects as such, it improves the tolerance to subsequent high ammonia exposure through priming mechanisms in ammonia excretory transcriptional processes.

A10.30 DISRUPTION OF ION BALANCE FOLLOWS DEATH IN COLD STRESSED TROPICAL SHRIMP (*MACROBRACHIUM ROSENBERGII*)

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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The tolerance to low temperature exposures is of critical ecological importance to ectotherms and it is therefore also of interest to understand the physiology determining their critical thermal tolerance. Here we evaluate two popular physiological models of low

temperature tolerance in arthropods using the tropical freshwater shrimp *Macrobrachium rosenbergii*. One proposed model for the thermal limits of aquatic arthropods is the oxygen- and capacity-limited thermal tolerance (OCLTT), where the thermal limit is set by a failure of oxygen transport or aerobic metabolism beyond a temperature threshold. We are currently testing this model by investigating whether hypothermia compromises oxygen availability (causing a decrease in blood oxygen content) and/or if aerobic metabolism is compromised at low temperature (causing a rise in anaerobic metabolites). An alternative physiological explanation for the lower thermal tolerance has recently been described for a number of insect species where cold exposure disrupts ion balance through a progressive rise in extracellular [K⁺] that causes onset of cold injury and death. However we found from muscle and hemolymph samples that a disruption of ion balance did only occur after the onset of cold injury indicating that failure to maintain ion balance is not the cause, but rather a consequence of death.

A10.31 DELIMITATION OF THE TIME SINCE DEATH BY ANALYSIS OF POST MORTEM MUSCLE DEGRADATION

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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Estimation of the time since death plays an indispensable role in the resolution of criminal cases. The awareness of the precise time of death gives many further explanations about the circumstances of death, validates a witness's statement, assesses alibis and thus narrows the field of suspects. There are numerous methods proposed for time of death estimation, but just a few of them achieved practical importance. The available methods are still very inaccurate, limited to short post mortem periods and are also highly dependent on several influencing factors (e.g. temperature, humidity, cause of death...). Therefore it is necessary to improve and expand the range of methods substantially. In this study we take advantage of the post mortem degradation process of human skeletal muscle and correlate specific appearing degradation products with certain post mortem time periods. For this purpose we used SDS-PAGE and Western blotting to determine the degradation process of selected proteins (troponin T, desmin, tropomyosin) in muscle samples of 40 forensic cases. Additionally, casein zymography was performed for analysis of calpain activity. We could demonstrate predictable characteristic alternations in the protein profiles until the first 10 days post mortem. Further, we also analyzed influencing factors (temperature, BMI, age) that are likely to affect the degradation process. The obtained results show clearly the potential of post mortem protein degradation for the estimation of the time since death.