The Interaction between environmental temperature and metal toxicity tolerance in zebrafish (Danio rerio)

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1. Introduction.

Zebrafish has become a valuable vertebrate model organism in a wide range of biological research including ecotoxicology. Although there are several studies on metal toxicity in zebrafish, investigations on the interaction between thermal background of the organism and metal uptake and toxicity are rare.

Toxicology studies are mainly performed in standardized conditions, most often at the optimal rearing temperature. However, environmental temperature varies considerably in field situations, such that it seems evident to include the effect of temperature on chemical toxicity into ecotoxicological assessments (Sokolova and Portner, 2007).

2. Materials and methods

As shown in Table1, two main thermal scenarios were defined:**1-metal exposure subsequent a thermal challenge:** this scenario included acute and chronic thermal challenges in 5 different temperatures subsequently after the thermal challenge fish were introduced to metal contaminated water for 10 days in 28°C. **2-metal exposure in a constant thermal challenge**: After acclimatizing fish to 5 different temperatures for 4 weeks they were exposed to metals for 10 days while the thermal challenge continued.

	Description	*Treat*	*Thermal Challenge*	*Metal exposure*
Scenarios	Metal and temperature as separate stressors	Acute	17, 22, 25, 32 and 34°C for 12 and 96 hours	Cu 75 µgr/L or Cd 25 µgr/L or Cu 75+ Cd 25 µgr/L In 28°C
		Chronic	17, 22, 25, 32 and 34°C for 4 weeks	Cu 75 µgr/L or Cd 25 µgr/L or Cu 75+ Cd 25 µgr/L In 28°C
	Interaction of thermal and metal pollution stress	Acclimatization	17, 22, 25, 32 and 34°C for 4 weeks	Cu 75 µgr/L or Cd 25 µgr/L or Cu 75+ Cd 25 µgr/L In 17, 22, 25, 32 and 34°C

Table 1: Overview of the applied thermal challenges and metal toxicity scenarios.

The condition of the fish was monitored during the experiment, including behavioural and physiological metrics and also water quality monitoring. At the end of the exposure period, whole body metal and essential electrolyte concentrations were determined using ICP-OES or ICP-MS.

3. Results and discussion

3.1. Metal uptake

The results indicated that the thermal scenarios as well as the metal exposure concentrations and their interaction significantly influenced the Cu and Cd body burden. Although Cu was much more toxic than Cd, Cu and Cd together showed a large synergistic effect. A low temperature shock appears to be protective while a high temperature shock increases sensitivity. Whereas, acclimation to warm temperatures increase the tolerance against metal toxicity in comparison to acclimation to cold temperatures.

The results of whole body metal analysis showed that the metal burden in heat shocked fish is significantly higher than the cold shocked ones. While, the fish which acclimatized to cold temperature show a higher metal level in comparison to the fish which acclimatized to a warm temperature (Figure 1).

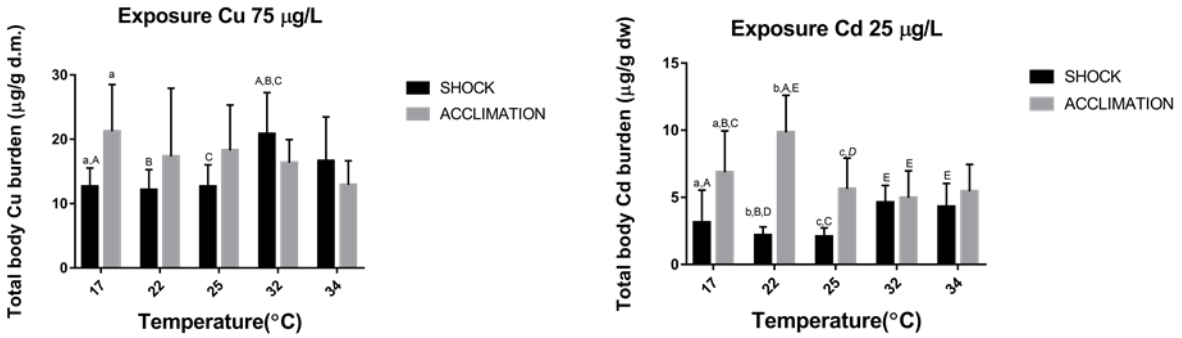

Figure 1: By increasing the shock temperature metal uptake increases. Whereas, the total body metal burden in cold acclimatized fish is significantly higher than warm acclimatized fish (data for surviving fish± SD). Same letters show significant differences among groups. (Two way Anova).

3.2. Ionic regulation

Considering the major role of electrolytes in keeping the body homeostasis constant we wanted to figure out the importance of the electrolyte balance potentially making the difference between survival or failure under multi stress conditions?

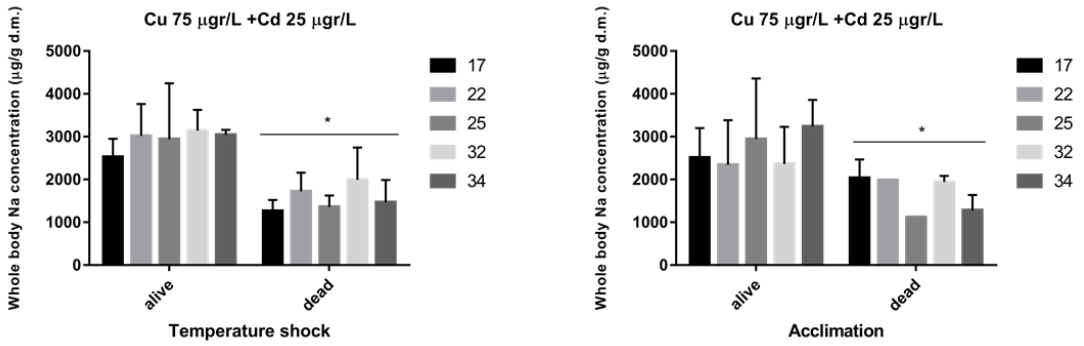

Figure 2: The whole body sodium level is significantly lower in dead fish in comparison to live fish. Both shock and acclimation scenarios show the same effect. (Two way Anova)

Investigation of the electrolyte levels (Na, K, Ca and Mg) showed a significant drop in sodium levels between the alive and dead fish (Figure 2). Such an effect was not observed for the other major cations.

4. Conclusions

The results showed that thermal prehistory and environmental temperature play an important role in determining zebrafish tolerance against metal toxicity. In shock scenarios the sensitivity increases by increasing the temperature. While, in acclimation scenarios warm temperatures appear to be protective in comparison to cold temperatures. Analysis of the major electrolytes composition of the body shows that survival strongly depends on the capacity to maintain Na balance as an overall indicator of the capacity to maintain homeostatic balance or get into a failure scenario.

5. References

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