The annual killifish *Nothobranchius furzeri* as a new model in long-term ecotoxicological research

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1. Introduction

Given the obligation to perform long-term tests on vertebrates in ecotoxicological risk assessment[1] and the high cost in terms of labour, money and time associated with such tests on current models, there is a need for new and efficient vertebrate model species to study chronic and multigenerational toxicity. *Nothobranchius furzeri*, the turquoise killifish, has two characteristics which make it into a good candidate model organism for long-term freshwater ecotoxicological tests. First of all, the species is characterised by a very short life cycle of ±12 weeks and second, it produces drought-resistant eggs which can remain viable in a state of dormancy up to several years[2]. While the former characteristic facilitates multi-generational testing over a short time-span, the latter removes the need for a continuous culture of test organisms and enables synchronised exposure of the test population through controlled hatching.

In order to test its validity as a new model organism, we tested the acute and chronic sensitivity of *N. furzeri* to several pollutants. In these exposure experiments, we wanted to determine the sensitivity of the species to a range of well-studied toxicants with different modes of action and compare its sensitivity range to that of current fish models. Furthermore, as exposure times may be much longer in natural compared to laboratory settings and effects may only emerge in later stages of an organism's life cycle[3], we also studied long-term effects of sublethal concentrations of toxicants in an environmentally relevant context. Although we will present data on exposure to several reference toxicants, the main focus of the presentation will be on chronic, sublethal effects of copper exposure.

2. Materials and methods

To perform acute toxicity exposures, we selected commonly found and well-studied toxicants from different toxicological classes. Cadmium and copper were chosen as a metal, 3,4-dichloroaniline as an aniline and chlorpyrifos as a organophosphorous pesticide. Forty-eight hours post hatching, each larva was transferred to a 0.5-L jar and subjected to a specific toxicant treatment in a semi-static setup. Mortality was monitored every 12 hours as a binary response (0=dead, 1=alive). Concentration-response curves (Fig. 1) were drawn and LC50 values were estimated at 24 hour intervals. For reference, we performed a literature search of comparable short-term exposure set-ups in other fish species.

The long-term exposure experiments followed the same protocol during the first two weeks after which fish were transferred to 2L jars. Life history traits including size and age at maturation, fecundity and life span were assessed for all fish surviving to the reproductive phase. Total length was measured weekly during the first 5 months and every two weeks after growth had levelled off in all treatments (from week 19 onwards). At the cellular level, we measured the bioaccumulation of copper in all fish.

3. Results and discussion

3.1. Acute sensitivity

The results of our experiments reveal different response patterns for each of the toxicants, dependent on the mode of action. As mortality in the control conditions was below 10%, the fish model appeared not to suffer any stress from our handling method. In general, and on the basis of applied acute test concentrations, the sensitivity of *N. furzeri* appears to be comparable to or higher than the sensitivity of other model species.

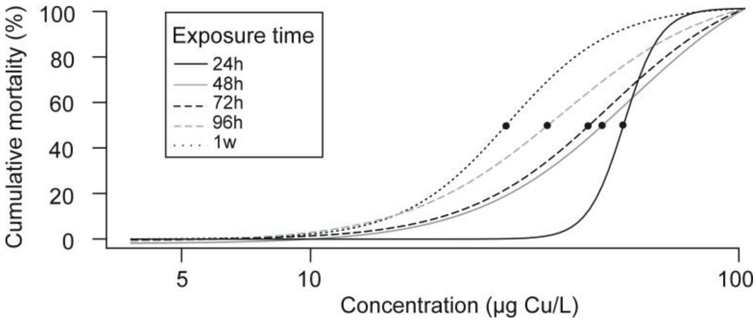

Figure 1: Dose-response curves showing cumulative mortality against concentration (in µg Cu/L). Dots indicate LC_{50} values for every time point.

3.2. Chronic sensitivity to copper

N. furzeri showed an increased lethal damage, decreased life span, slower maturation and reduced fecundity (Fig. 2) when chronically exposed to copper concentrations of ≥10.27 µg Cu/L. Our results revealed delayed mortality effects of the copper exposure which would not be observed in standard toxicity tests that typically only last for 96 h. Furthermore; it should be mentioned that at a concentration of 6.68 µg Cu/L *N. furzeri* showed an increased lifespan and reproduction, what could be the result of a toxicant induced metabolic increase (hormesis). In general, we observed a damage and repair mechanism, with fish either succumbing to the exposure during the first weeks, or developing defence and copper-excreting mechanisms to endure exposure on the long term, potentially trading off with fitness.

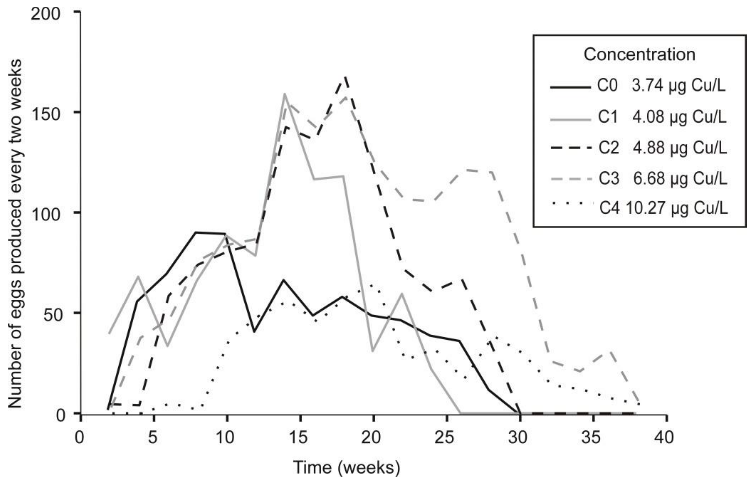

Figure 2: Sub-lethal endpoints such as egg laying patterns may reveal stress, providing valuable insights on the general condition of fish in a specific treatment.

4. Conclusions

Nothobranchius furzeri is a suitable new model for ecotoxicological purposes. Since the species appears to be more sensitive compared to traditional model species and has a short generation time it is especially suited for long term exposure and multi-generational assays, which are considered highly relevant.

References

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