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Interspecific variation in tolerance of tilapia yolk sac fry (Family: Cichlidae) to copper stress

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Abstract

Tolerance to pollutant stress could be related to modes of life of species. Although differences in mode of life may or may not be brought about by stress, such differences could be adaptive in terms of pollutant resistance. In the present study, the tilapia yolk sac fry were exposed to acute copper stress in order to determine whether there is a general tolerance response among faster developing substrate spawning and slower growing mouth brooding tilapia yolk sac fry. To predict the possible underlying mechanism to withstand metal stress, early life history growth traits, such as specific growth rate and yolk utilizing efficiency, were measured in tilapia yolk sac fry under non-stress conditions. The six tilapia species used in this study were *Oreochromis niloticus, Oreochromis mossambicus, Oreochromis aureus* as mouth brooding species and *Sarotherodon galilaeus, Tilapia rendalli and Tilapia zillii* as substrate spawning species.

There was a significant ($p<0.05$) inter-specific variation in the response to acute copper stress among the species tested. The yolk sac fry of mouth brooding species were consistently more sensitive than those of the substrate spawning species. The inter-specific variation in mean specific growth rate (df = 5,12; F = 999.99; $p<0.05$) and yolk utilizing efficiency for the period from hatching to maximum body weight attainment (df = 5,12; F = 21.196; $p<0.05$) were significantly different.

The response and the rank order of tolerance to copper between the six species tested reflect a difference in general stress response between the two groups of substrate and mouth brooding tilapia, which may be related to differences in modes of life.

Introduction

Fish species can tolerate pollutant stress by specific modes of life. Although differences in modes of life may or may not be brought about by stress, such difference could be adaptive in terms of pollutant resistance. In a previous study (Siriwardena *et al.* 1993), it was hypothesised that in a stressful environment, faster developing species may have a fitness advantage over other species by minimising the duration of sensitive early developmental stages to stress factors such as chemicals. This hypothesis was explored by examining interspecific variation in tilapia yolk sac fry responses to acute cadmium stress. The most recent classification of the tribe tilapini recognizes three genera differing in reporductive behaviour, and it has been suggested that the more primitive substrate spawners of the genus *Tilapia* have given rise to two distinct groups of mouth brooders, the genera *Oreochromis* and *Sarotherodon*. Mouth brooders produce much larger yolkier eggs than substrate spawners and the resulting offspring of the former have a more protracted early development period than those of the latter.

Life history traits could be used to predict how animals are likely to respond to different levels of stresses as they are expected to be correlated with the availability of metabolic energy (Hoffman & Parsons 1989). In the present study, yolk sac fry of six tilapia species were exposed to copper stress in order to determine whether there is a general tolerance response among tilapia to metal stress. Early life-history growth traits were also measured in tilapia yolk sac fry under non-stressed (control) conditions to predict the possible underlying mechanism to withstand metal stress.

Materials and methods

Test organism and testing system

The six tilapia species used in this study were *O. niloticus*, *O. mossambicus*, *O. aureus*, *S. galiaeus*, *T. rendalli* and *T. zillii*. Six-day old yolk sac fry of each *Oreochromis* and *Sarotherodon* species and two-day old yolk sac fry of *Tilapia* species were obtained from incubation systems in the tropical aquarium of the Institute of Aquaculture at the University of Stirling. Six day old yolk sac fry of mouth brooders and two-day old yolk sac fry of substrate spawners can be considered as in equivalent developmental stages (Galman & Avtation 1989, Rana 1988). Fry of each species used in the experiment were derived from a single clutch. A total of 450 fry, in groups of 30 were divided among fifteen 260 ml exposure chambers of a flow-through system filled with ASTM (1980) soft dilution water. This system had sixteen fully independent channels. Each channel comprised a 2.5 litre glass reservoir containing the exposure solution, which was pumped via a Watson-Marlow fixed speed peristaltic pump into a 260 ml Nunclon tissue culture flask exposure chambers at a flow rate of 1.2 ml min^{-1}. The loading rate used within each channel of the system was 0.104g l^{-1}. After a 24 h acclimation period, fry in exposure chambers were randomly allocated to one of five triplicated treatment levels (including the control). Each exposure chamber was checked and the number of fry responding was recorded at 24 h intervals for a period of 96 h. Same experimental design was repeated for each species.

Preparation of test concentrations

Test concentrations were prepared by serial dilution of a 20 mg l^{-1} copper stock solution prepared by dissolving 53.7 mg of Analar grade dihydrated cupric chloride in one litre of ASTM dilution water. Copper levels of the test concentrations were determined by atomic absorption spectrophotometry after 100 ml sample of each concentration was acidified with 1% Aristar nitric acid and frozen in clean plastic bottles.

Acute toxicity tests

All acute toxicity tests were performed according to established guidelines (OECD 1993). Initial range finding experiments were carried out for copper to determine 5 test concentration of 0. 1. 10. 100. 1000 μ g l^{-1}. During the range finding test. if the lethal levels of copper for any of the species fell outside the range or control mortality exceeded 20%. the test was repeated using a different toxicant range. The test end point was immobilization. based on response to gentle prodding with a glass rod.

Early life-history growth traits under non-stressed (control) conditions

At hatching. yolk sac fry from an individual egg clutch were stocked in 15 exposure chambers at a density of 40 yolk sac fry per chamber. To eliminate the influence of reduced stocking density. three exposure chambers were randomly selected and all fry in chambers were sampled at 3. 6, 9. 12 and 15 days post-hatch. The fry were killed in benszocaine. The bodies of half of the each sample were dissected from their yolk sacs (if present) under a dissecting microscope and the bodies and fry (bodies + yolk. if present) were weighed after removing excess surface moisture with absorbent paper. The samples were oven dried overnight at 60°C. The dried samples were then cooled in a desiccator to room temperature and reweighed to an accuracy of 0.0001g on a top pan balance. Similarly. the initial body weight and whole weight of fry at hatch obtained from the same egg clutch were determined. This procedure was repeated in triplicate. These data were used to calculate the specific growth rate and yolk utilisation efficiency.

Statistical analyses

A proportional mortality response based on measured actual rather than nominal concentrations was calculated using a standard probit procedure (Finney 1971) to estimate the 50% acute concentration (EC 50). Goodness of fit for each data set to the probit model was assessed by comparison with critical Chi-square value ($P = 0.05$). One way ANOVA and Tukey HSD multiple range tests (Zar 1984). were used to compare the values of specific growth rates and yolk utilization efficiencies of different species. Correlation coefficient between the stress tolerance and early life history growth traits was also calculated. Where appropriate. data were first normalised using an arcsine transformation.

Results

Interspecific variation in sensitivity to acute metal stress

There were significant interspecific variations for acute tolerance to copper. In all cases. concentration response data fitted the probit model adequately ($P < 0.05$). The predicted LC_{50} value, together with its calculated 95 % confidence limits. for each species is displayed in Figure 1 as a normal probability density function (midpoint = EC 50: kurtosis = intensity of the response). Two things are apparent from the density functions given in Figure 1. First. there was significant ($P < 0.05$) variation in the response to copper among the species tested. of almost an order of magnitude of (from70.55 to 439.30 μg l^{-1}). The yolk sac-fry of mouth brooding species. as predicted. were consistently more sensitive than those of the substrate spawners. Second the response of individuals within the species tested was remarkably consistent giving extremely steep responses (hence the leptokurtotic, or 'spiked' appearance of the density functions). The correlation coefficient showed that there was a clear concordance ($r > 0.999$; $p > 0.00$) in the rank order of species response for the two toxicants.

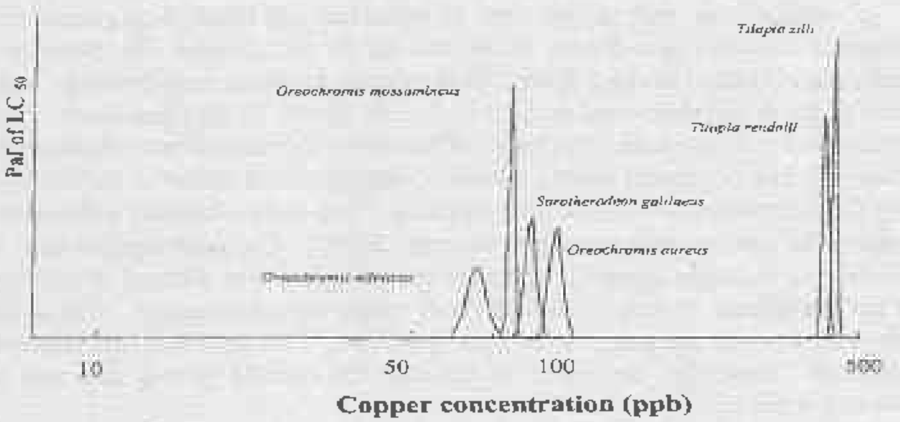

Figure 1 Interspecific variation in lethal responses of 6 day old Tilapia yolk-sac fry to copper Responses are expressed as probably density functions of the 96 h LC_{50} (mean and 95% confidence limit)

Early life-history growth traits under non-stressed (control) conditions

The time taken to attain maximum weight, maximum length and to complete utilization of yolk in mouth brooding species was about twice as that of substrate spawning species. The mean growth characteristic of yolk sac fry of the six species studied are given in Table 1.

The specific growth rate and yolk utilization efficiency for the period from hatching to maximum body weight attainment of the tested species are shown in Table 2. The interspecific variation in mean specific growth rate and yolk utilization efficiency for the period from hatching to maximum body weight attainment were significantly different $P<0.05$).

In general, significantly ($P<0.05$) higher specific growth rate and yolk utilization efficiency values were obtained for yolk sac fry from substrate spawners than those of mouth brooders. The specific growth rate and yolk utilization efficiency values between the substrate spawnwers, *T. rendalli* and *T. zillii*, were not significantly different ($P>0.05$). The mean values for specific growth rate and yolk utilization efficiency of *O. niloticus* yolk sac-fry from hatching to maximum body weight attainment was significantly lower than those of other mouth brooding tilapia yolk sac fry (P>0.05).

Table 1. Growth characteristics of fry of the six tilapia species tested

	Species					
	O. niloticus	*O. mossambicus*	*O. aureus*	*O. galilaeus*	*T. zillii*	*T. rendalli*
Dry body weight of fry at the beginning of exposure Mean ± SD (mg)	1.45 ± 0.025	1.04 ± 0.012	1.37 ± 0.015	1.14 ± 0.046	0.20 ± 0.007	0.21 ± 0.005
Dry body weight of fry at the beginning of exposure as % of maximum attained	80.50	77.68	76.53	74.27	58.97	58.00
Age at end of yolk sac stage (days)	12	12	15	15	6	6
Age at the time of maximum body weight (days)	[illegible]	9	9	9	5	5

A significant correlation between copper EC_{50} values and specific growth rate (r=0.997, P <0.001) and between copper EC_{50} values and yolk utilization efficiency (r=0.986, P<0.01) was observed.

Table 2. Mean values for specific growth rate and yolk utilization efficiency of the fish species tested. (In each column values indicated by the same letter are not significantly different from each other)

Species	Specific growth rate (%)	Yolk utilization efficiency (%)
O. niloticus	26.0^{a}	58.5^{d}
O. mossambicus	29.5^{b}	62.5^{e}
O. aureus	29.4^{b}	66.0^{e}
S. galiaeus	29.2^{b}	63.8^{e}
T. rendali	39.2^{c}	70.2^{f}
T. zilli	39.7^{c}	70.3^{f}

Discussion

The results indicate that interspecific differences in sensitivity of yolk sac fry of tilapia species are significant for copper. The variability in response and the rank order of tolerance of tilapia species observed for copper were similar to those for cadmium (Siriwardena *et al.*, 1993). This suggests that the mode of action of and/or the mechanism of detoxification for cadmium and copper could be similar. Contrary to this observation i.e., lack of concordance in acute tolerance of *Daphnia magna* metals have been recorded by Baird *et al.* (1991). The intraspecific variation of *Daphnia* in response to metals was generally within one order of magnitude, with the notable exception of cadmium which varied over three order of magnitude (Barid *et al.* 1991). Baird *et al.* (1991) attempted to attribute this exceptional response to cadmium to its non-essential metal category when compared to the other essential metals tested. In contrast, in the present study, the inter-specific variation of tilapia response to copper was around an order of magnitude of six suggesting a general response to acute metal stress. Therefore, the observed variation in interspecific tolerance to copper acute stress in the present study suggests the underlying mechanism may not be compound-specific genetically based pre-adaptation.

The observed differences between mouth brooders and substrate spawners may reflect a difference in general stress response between the two groups, which may be related to differences in their modes of life

Proposed models of stress tolerance (Sibly & Calow 1989) suggest that the existence of genetically based differences in growth may account for genetically based differences in stress tolerance. The efficiency of transforming yolk to body tissue would give an indication of how effectively resources are allocated for growth and other metabolic activities. High growth rate and yolk utilization efficiency indicate that less resources are used for maintenance than for growth. Thus, higher growth rates may be indicative of reduce energy investment for maintenance compared with growth, hence trade-off could be taking place between growth and maintenance to meet the higher maintenance cost under stress conditions. This may be the reason for higher tolerance recorded for faster growing yolk sac fry of substrate spawners than that of slower growing yolk sac fry of mouth brooding species.

In conclusion, there were significant differences in acute tolerance between the tilapia species tested for copper. Moreover, a concordance in response to copper and cadmium was observed, suggesting that the observed differences may be due to differences in the modes of life between mouth brooding and substrate spawning species. A reduction in the exposure period of sensitive developmental stages of early life stages could cause a reduction in uptake, and hence increase tolerance. An increased body burden for copper in the relatively slow developing mouth brooder sac fry could be expected as the sensitive early development stages will be exposed for longer periods than the fast growing sac fry of substrate spawners. In order to test this hypothesis further, it will be necessary to demonstrate differences in the metal uptake and its partitioned body burden between the two groups of fishes. Under non-stressed conditions, the yolk sac fry of substrate spawning and mouth brooding species showed significant ($P<0.05$) differences in their patterns of growth and these differences were found to be correlated with the tolerance capabilities of the two groups. The correlation between tolerance capability and growth performance suggests that the tolerance capability may be related to the energy investment on maintenance rather than on growth. As such, an intraspecific variation in tolerance response to metal stress may be expected in these tilapia groups due to differences in size and nutritional status among the member of the same species.

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