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Title: Parental stress-coping styles affect the behaviour of Rainbow trout *Oncorhycus mykiss* (Walbaum) at early developmental stages

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Abstract

This work examines behaviour responses in yolk sac fry originating from strains selected for high (HR) or low (LR) plasma cortisol response to a standardised stressor. The results shows that yolk sac larvae originating from the HR strain is more sensitive to environmental stressors, in that they showed a shorter reaction time to low oxygen levels. Previous studies on adult and juvenile individuals from these strains have demonstrated a number of correlated physiological and behavioural differences. In yolk sac larvae growth and development depend mainly on internal factors, which suggest that at least some aspects of stress coping styles are inherent to the individual, before factors such as exposure to social experience or variable access to food resources could modify behavioural strategy.

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Introduction

Differing behavioural strategies to cope with situations which challenge the fitness of an individual have been suggested to maintain genetic variation in a population (Korte *et al.*, 2005). These behavioural strategies correlate with consistent physiological traits, and have been referred to as stress coping styles (Koolhaas *et al.*, 1999), behavioural syndromes (Sih *et al.*, 1985), temperaments (Boissy, 1995; Clarke & Boinski, 1995) or animal personality traits (Buss, 1991; Gosling, 2001). Both genetic and environmental factors [e.g. social interactions and previous exposure to stress] contribute to extensive inter-individual variation in how stressful experience affects behaviour and physiology (Carere *et al.*, 2005; Frost *et al.*, 2007; Korzan & Summers, 2007). The tight relationship between stress coping style and social stress is demonstrated both by the influence that previous social interactions have on physiology and aggressive behaviour (Höglund, *et al.*, 2001;

Øverli *et al.* 2004b; Summers *et al.* 2005), and by how the capacity to react to stressful conditions with adaptive neuroendocrine responses predicts social position (Korzan *et al.*, 2006; Øverli *et al.*, 2004a).

Since most of the studies of behavioural traits associated with stress coping styles have been done on individuals with social experience, studies of socially naïve individuals could provide information of the genetic component of an individuals stress coping strategy, verifying the heritability of these traits. Furthermore, during yolk sac absorption, the growth and developmental rate of fish are mostly dependent on internal resources (Jobling, 1985). Effects of social interactions and current food supply are thus less important in yolk sac fry than in juvenile and sexually mature fish, making fish in early developmental stages a promising model for investigating the genetic impact on stress coping strategy. However, very little is known about the presence and expression of different stress coping styles in these developmental stages in fish.

The present study was designed to investigate effects of parental stress coping style on behaviour of rainbow trout at the yolk sac stage (*Oncorhyncus mykiss*). Early studies indicating the presence of individual stress coping styles in rainbow trout reported pronounced variation in the response to reduced environmental O_2 levels (vanRaaij *et al.*, 1996). Hence, it is of particular interest to investigate whether variation in avoidance behaviour in response to low O_2 is an acquired or inherited response. This question was addressed by exposing larvae, originating from strains that have previously been demonstrated to have contrasting stress coping styles (Øverli *et al.*, 2007; Øverli *et al.*, 2005), to low oxygen levels. The results demonstrate that strain origin affected behaviour during exposure to low oxygen levels, indicating that parental stress coping style affect behaviour in early developmental stages in fish.

Material and methods

Gametes were collected from sexually mature adult fish of the F3 generation from two rainbow trout strains (HR and LR), selected for divergent stress responsiveness to a standardized stressor (for details see (Øverli *et al.*, 2007). Fertilization was performed 27 December, 2005, at Solbergstrand research station. To minimize inbreeding depression, allelic variation in 6 micro-satellite markers were considered, when the 8 HR and 10 LR F4-families were formed. Stripping and fertilization was performed with standard methods. Eggs and larvae were incubated at 6,5-7,5 ^{o}C. Experiments were performed on mixed batches of these families.

Behaviour of isolated yolk sac larvae was studied at 550 degree-days after fertilization in glass boxes (50 mm deep, 10 mm wide and 80 mm high) containing water at 7 ^{o}C and either 100, 35 or 10% O_2 saturation. The required levels of oxygen saturation were obtained by bubbling N_2 while monitoring dissolved O_2 concentrations using an O_2 electrode (Oximeter 323A, WTW, Weilheim, Germany). One individual was placed in each box, whereupon the boxes were sealed with a glass lid. During each experiment, the behaviour of two LR and two HR larvae was recorded simultaneously on video during exposure to water with the same O_2 saturation level, starting 2 minutes after being inserted in glass boxes in random order. Video tapes were analysed for time to the initiation of avoidance swimming, defined as the time point at which fry first moved more than half its body length in one continuous movement. Time to the expression of avoidance behaviour was set to 450 sec if the fry did not move within 7.5 minutes of the start of the test period. In total, eight larvae from each strain were filmed during exposure to each treatment (10, 35 or 100 % O_2 saturation).

Data are presented as mean +/- standard error of mean. Data on time to avoidance were log-transformed and subjected to a two way analysis of variance (ANOVA) with parental

stress responsiveness (HR and LR) and oxygen saturation as independent variables, followed by the Tuckey honest significant test (HSD) posthoc test (Statistica 5.1, StatSoft Inc.).

Results

The two way ANOVA indicated that lag time to expressing avoidance behaviour was significantly affected by both parental stress coping style ($F_{1,42}$=4.88, P<0.05), oxygen saturation level, ($F_{2,42}$=16.8, P<0.001), and an interaction between these two factors ($F_{2,42}$=3.69, P<0.05). The effect of reduced O_2 levels was strongest in HR larvae, where time to avoidance differed significantly between 10 % and 35 % O_2 saturation (P<0.001), as well as between 10% and 100% O_2 saturation (P<0.001; Fig 1). In LR larvae, this relationship between was reflected in a non significant trend for shorter lag time at reduced O_2 levels; 10 % compared to 100 % O_2 saturation (P>0.05) and 35% compared to 10 % O_2 saturation (P>0.05; Fig 1). Latency to express avoidance behaviour differed significantly between HR and LR larvae exposed to 10 % oxygen levels (P<0.05), with HR larvae showing the shortest response times (Fig 1). There were no significant differences in lag time to express avoidance behaviour between the HR and LR line at 35% (P>0.05) or 100 % (P>0.05) O_2 saturation, or between 35 % and 100% O_2 saturation within the HR (P>0.05) or the LR (P>0.05) larvae.

Discussion

In the present study, decreased oxygen levels resulted in a shorter lag time for the initiation of avoidance behaviour in both the HR and LR strains. Furthermore, in the lowest oxygen saturation (10 %) the HR larvae initiated this behaviour earlier than the LR larvae.

Previous studies of adults and juveniles originating from the strains selected for high (HR) or low (LR) plasma cortisol response to confinement stress, demonstrate a number physiological and behavioural differences between the lines when challenged by a stressor

(reviewed by; (Øverli *et al*., 2005). Behavioural studies suggest that increased locomotor activity is a general characteristic of the stress response in the HR strain (Øverli *et al*., 2005; Schjolden *et al.,* 2005). Different behavioural responses to hypoxic conditions have been shown to correlate with other physiological and behavioural traits, describing an individuals' stress coping style in juvenile and adult salmonid fish (Brelin, *et al.*, 2005; vanRaaij *et al.,* 1996). Hence, it seems likely that the different responses to hypoxic conditions exhibited by HR and LR larvae in the present study, reflects parental stress coping style.

The heritability of different stress coping styles has been suggested to maintain genetic variation in a population (Korte *et al.,* 2005). However, theoreticians suggest that the behavioural output during a challenge should be plastic in order to adopt a behaviour which is beneficial for an individual in an actual situation (Dall *et al.,* 2004). In addition to genetic factors (Koolhaas *et al.,* 1999; Koolhaas *et al.*, 2007; Øverli *et al.*, 2007), environmental factors and experience, such as social stress, modulate individual stress coping style (Carere *et al.,* 2005; Frost, *et al*., 2007; Korzan and Summers, 2007). The present study demonstrates the presence of divergent stress coping styles during the yolk sac stage in rainbow trout. These differences reflect the respective parental stress coping style, and are present before exposure to social stress or other environmental inputs, such as variable or insufficient food supply. This suggests a rather strong heritability in this species. The mechanisms for this inheritance have to be further investigated. In the present study maternal effects, such as egg size or yolk composition was not investigated. However, earlier studies of these strains do not indicate any difference in egg size (Pottinger and Carrick, 2000). If other maternal effects, such as hormone deposition in eggs and/or yolk composition, affect individual stress coping style needs further studies.

In conclusion, the present study demonstrates that decreased oxygen levels induces a shorter lag time for expressing avoidance behaviour in rainbow trout yolk sack larvae.

Furthermore, the observation that yolk sack larvae originating from the HR strain showed stronger avoidance to hypoxic conditions than the LR strain, suggest that strain differences in behaviour are expressed before social experience or other environmental factors could modify an individual's stress coping style.

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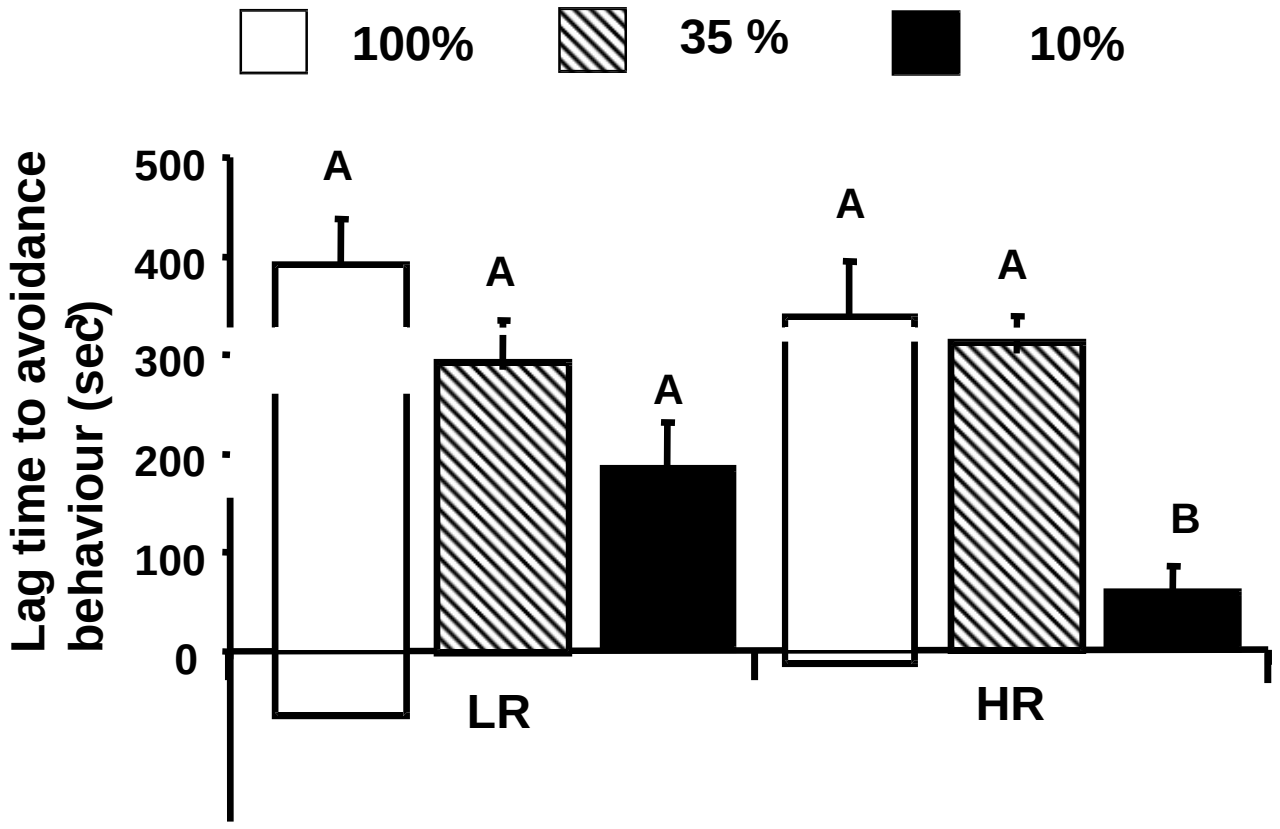
238 Fig 1. Latency to express avoidance behaviour in rainbow trout yolk sack larvae exposed to water
239 with 100, 35 and 10 % O_2 saturation at 550 day degrees after hatching. The larvae originated from
240 parents selected for high (HR) or low (LR) stress responsiveness. Behaviour responses were studied
241 in isolated larvae. Eight LR and HR larvae were exposed to each oxygen saturation level. Different
242 letters indicates significant differences ($P<0.05$)
243

Figure


100%
35 %
10%
Lag time to avoidance behaviour (sec)
500
400
300
200
100
0
A
A
A
A
A
B
LR
HR