Parental stress-coping styles affect the behaviour of rainbow trout *Oncorhynchus mykiss* at early developmental stages

E. Höglund*, H.-M. Gjøen‡, T. G. Pottinger§ and Ø. Øverli‡

*Department of Marine Ecology and Aquaculture, Danish Institute for Fisheries Research, Technical University of Denmark, North Sea Center, P. O. Box 101, DK-9850 Hirtshals, Denmark, ‡Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P. O. Box 5003 As, Norway and §NERC Centre for Ecology and Hydrology Lancaster, Lancaster Environment Centre, Bailrigg, Lancaster LA1 4AP, U.K.

(Received 3 January 2008, Accepted 13 August 2008)

This work examined behavioural responses in yolk-sac rainbow trout *Oncorhynchus mykiss* larvae originating from strains selected for high (HR) or low (LR) plasma cortisol response to a standardized stressor. The results showed that yolk-sac larvae originating from the HR strain were 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 demonstrated a number of correlated physiological and behavioural differences. In yolk-sac larvae, growth and development depended mainly on internal factors, which suggest that at least some aspects of stress-coping styles are inherent to the individual, before factors such as social experience or variable access to food resources could modify behavioural strategy.

Key words: development; fish; heritability; personality; plasticity; teleost.

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 interindividual 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 of previous social interactions on physiology and aggressive behaviour (Höglund et al., 2001; Øverli et al., 2004a; Summers et al., 2005), and by how the capacity to react to stressful conditions with adaptive neuroendocrine responses predicts social position (Øverli et al., 2004b; Korzan et al., 2006).

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 about the genetic component of an individual’s stress-coping strategy, verifying the heritability of these traits. Furthermore, during yolk-sac absorption, the growth and developmental rate of fishes are mostly dependent on internal resources (Jobling, 1985). Effects of social interactions and current food supply are thus less important in yolk-sac larvae than in juvenile and sexually mature fishes, making fishes in early developmental stages a promising model for investigating the genetic effect on stress-coping strategy. Very little is known, however, about the presence and expression of different stress-coping styles at these developmental stages in fishes.

The present study was designed to investigate effects of parental stress-coping style on behaviour of rainbow trout Oncorhynchus mykiss (Walbaum) at the yolk-sac stage. Early studies indicating the presence of individual stress-coping styles in O. mykiss reported pronounced variation in the response to reduced environmental oxygen levels (van Raaij et al., 1996). Hence, it is of particular interest to investigate whether variation in avoidance behaviour in response to low oxygen 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., 2005, 2007), to low oxygen levels.

**MATERIALS AND METHODS**

Gametes were collected from sexually mature adult fish of the F3 generation from two O. mykiss strains (HR and LR), selected for divergent stress responsiveness to a standardized stressor (Øverli et al., 2005). Fertilization was performed on 27 December, 2005, at Solbergstrand Research Station. To minimize inbreeding depression, allelic variation in six micro-satellite markers were considered, when the 8 HR and 10 LR F4 families were formed. Stripping and fertilization were performed with standard methods. Eggs and larvae were incubated at 6.5–7.5°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°C and either 100, 35 or 10% oxygen saturation. The required levels of oxygen saturation were obtained by bubbling N₂ while monitoring dissolved oxygen concentrations using an oxygen 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 oxygen saturation level, starting 2 min 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 s if the fry did not move within 7.5 min 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% oxygen saturation).

Data are presented as mean ± s.e. Data on time to avoidance were log_{10}-transformed and subjected to a two-way ANOVA with parental stress responsiveness (HR and LR) and oxygen saturation as independent variables, followed by the Tukey honest significant difference test (HSD) post hoc test (Statistica 5.1; StatSoft Inc., Tulsa, OK, U.S.A.).

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 oxygen levels was strongest in HR larvae, where time to avoidance differed significantly between 10 and 35% oxygen saturation ($P < 0.001$), as well as between 10 and 100% oxygen saturation ($P < 0.001$; Fig. 1). In LR larvae, this relationship was reflected in a non-significant trend for shorter lag time at reduced oxygen levels: 10 compared to 100% oxygen saturation ($P > 0.05$) and 35 compared to 10% oxygen 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% ($P > 0.05$) oxygen saturation, or between 35 and 100% oxygen 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.

---

**Fig. 1.** Latency to express avoidance behaviour in *Oncorhynchus mykiss* yolk-sack larvae exposed to water with 100(□), 35(■) and 10(□) % oxygen saturation at 550 degree-days after hatching. The larvae originated from parents selected for high (HR) or low (LR) stress responsiveness. Behaviour responses were studied in isolated larvae. Eight LR and HR larvae were exposed to each oxygen saturation level. Different upper case letters indicates significant differences ($P < 0.05$).
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 of physiological and behavioural differences between the lines when challenged by a stressor (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 salmonids (van Raaij et al., 1996; Brelin, et al., 2005). 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). Theoreticians, however, 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, 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 & Summers, 2007). The present study demonstrates, the presence of divergent stress-coping styles during the yolk-sac stage in *O. mykiss*. 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. Earlier studies of these strains, however, do not indicate any difference in egg size (Pottinger & Carrick, 2000). If other maternal effects, such as hormone deposition in eggs and yolk composition, affect individual stress-coping style needs further study.

In conclusion, the present study demonstrates that decreased oxygen levels induces a shorter lag time for expressing avoidance behaviour in *O. mykiss* 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, suggests that strain differences in behaviour are expressed before social experience or other environmental factors could modify an individual’s stress-coping style.

This study was supported by the Norwegian Research Council.

References


