Consistent boldness behaviour in early emerging fry of domesticated Atlantic salmon (*Salmo salar*): Decoupling of behavioural and physiological traits of the proactive stress coping style

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ABSTRACT

Individual variation in the way animals cope with stressors has been documented in a number of animal groups. In general, two distinct sets of behavioural and physiological responses to stress have been described: the proactive and the reactive coping styles. Some characteristics of stress coping style seem to be coupled to the time to emerge of fry from spawning redds in natural populations of salmonid fishes. In the present study, behavioural and physiological traits of stress coping styles were compared two and five months after emergence in farmed Atlantic salmon (*Salmo salar*), using individuals with an early or late time to emerge. Initially, compared to late emerging individuals, early emerging individuals showed a shorter time to resume feeding after transfer to rearing in isolation. Resumption of feeding after isolation was suggested to be related to boldness behaviour, rather than hunger, in the present study. This observation was repeated five months after emergence, demonstrating behavioural consistency over time in this trait. However, in other traits of proactive and reactive stress coping styles, such as social status, resting metabolism or post stress cortisol concentrations, early and late emerging individuals did not differ. Therefore, this study demonstrates that boldness in a novel environment is uncoupled from other traits of the proactive and reactive stress coping styles in farmed salmonids. It is possible that this decoupling is caused by the low competitive environment in which fish were reared. In natural populations of salmonids, however, the higher selection pressure at emergence could select for early emerging individuals with a proactive coping style.

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1. Introduction

Individual differences in the way animals respond to a challenge are often referred to as behavioural syndromes, coping styles, temperaments or animal personalities [1], and have been documented in mammals [2], birds [3], reptiles [4], and fish [5]. In particular, behavioural syndromes are characterised by behavioural responses that are correlated across different situations [6], whereas the term stress coping styles is often used to describe suits of behavioural and physiological responses to challenges that are constant over time [2]. Such behavioural and physiological differences have been clustered into two characteristic responses, termed proactive and reactive stress coping styles [2]. Proactive individuals exhibit low glucocorticoid, high adrenaline release and a fight-flight response to a challenge, social dominance, routine formation and bold behaviour. Reactive individuals, on the other hand, show a freeze–hide cortisol dominated response to a challenge, social subordination, behavioural flexibility and a more shy behaviour [2,7,8].

Several studies have suggested that the degree to which different characteristics correlate to each other may depend on the context in which they are expressed and on the magnitude of the selection pressure [9–15]. For example, in sticklebacks (*Gasterosteus aculeatus*), bolder males were more aggressive towards other individuals when predation pressure was high, whereas this correlation was absent when the predation pressure was low [9]. Similar findings were observed in song sparrows (*Melospiza melodia*), where the correlation between aggression and boldness varied between populations adapted to human presence and populations in the wild [12]. Domestication of fish has also been shown to affect the relationship between behavioural and physiological traits [10,11,13,15]. Moreover, the relation between traits in an individual can be produced by both selection and by behavioural plasticity [9]. In rainbow trout (*Oncorhynchus mykiss*), for example, it has been demonstrated that the energetic status of an individual can affect the relationship between physiological and behavioural traits [16].

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Stress coping styles have been suggested to be related to the energy utilisation and metabolic rate of an individual [17]. In accordance with this, fast-growing individuals tend to have higher metabolic rates, show higher levels of aggression when fighting over food resources, and bolder behaviour while foraging in the presence of a predator [18]. In the common carp (*Cyprinus carpio*), individuals displaying a risk taking behaviour had higher metabolic rates and lower stress responsiveness, compared to individuals showing a risk avoidance behaviour [19]. A link between stress coping styles and metabolism may exist in salmonids, where individuals with higher metabolic rates grow faster and are most likely to become dominant, compared to individuals with a lower metabolic rate [20,21]. All these suggest that the proactive coping style is associated with a more costly strategy, whereas the reactive type is characterised by an energy conserving strategy, as suggested by Korte and co-workers [8].

In Atlantic salmon (*Salmo salar*), the time to emerge from a spawning redd may vary as much as 2 weeks [22 and references therein]. Early emerging fry exhibit several traits which are common with the proactive stress coping style, such as higher metabolic rate, social dominance, and a bolder behaviour [20]. Taken together, this indicates that timing of emergence in salmonids could be related to stress coping styles. However, it is still unknown if such differences are present in domesticated fish and if differences are maintained over time. Therefore, the aim of this study was to examine the relationship between emergence time and selected traits indicative of stress coping style in domesticated Atlantic salmon. Larvae were incubated in artificial spawning redds, which allowed fry to be sorted with respect to time to emerge. Two months later, resumption of feeding after isolation, standard metabolic rate, post stress cortisol concentrations, and social status were compared between fractions of early and late emerging fry. In addition, the aim was to investigate whether differences between early and late fractions of fish were consistent over time, and thus, differences detected in this first set of experiments were re-investigated five months after emergence.

2. Material and methods

2.1. Study material and experimental design

In November 2008, eggs from 144 families, originated from 144 females and 74 males, were stripped and fertilised over a period of 3 weeks. Families with different fertilisation dates were incubated at different temperatures in order to attain equal day-degrees after fertilisation (DDF). In February 2009, eyed eggs (347 DDF) were transported from Marine Harvest, Øyerhamn, Hardanger, Norway, to the research facilities of the Technical University of Denmark (DTU) at North Sea Centre in Hirtshals, Denmark. At DTU, batches of eggs from 100 of these 144 families (80–100 eggs per family) were randomly selected and were incubated separately.

Eggs were incubated in three batches, and each batch consisted of 1410 eggs from 48 different families. At 654 DDF, after all larvae were hatched, these three batches were inserted into three modified flat screen incubators. Inside the incubators, golf balls were placed to mimic natural gravel. In these incubators, larvae were sorted by time to emerge by flushing downstream to a collecting tank when they emerged from the incubator to search for food at the surface (Fig. 1). During the first 7 days after the larvae were inserted in the incubators, 5–20 individuals per incubator were collected daily. These individuals were re-introduced to the incubators. Thereafter, at 718 DDF, the number of individuals collected daily increased to 40–50 in all the incubators, and sorting of individuals with respect to emergence time started. After 17 days (at 870 DFF) almost all the individuals had left the incubators. During emergence, the daily water temperature was 8.9 ± 0.3 °C (mean ± s.e.m.).

During May–June 2009, two initial sets of experiments were carried out. In the first set of experiments, the time taken to resume feeding after isolation, standard metabolic rate (SMR) and whole body post stress cortisol concentrations after confinement stress were measured. From each of the early and late fractions, 19 individuals were randomly selected and isolated for 5 days in ten observation aquaria with a removable opaque dividing wall in the middle of the tank. Out of these selected fish, 8 individuals from each fraction were used as unstressed controls (see below). In the second set of experiments, 9 pairs of fish with a similar body mass (within pair difference < 5%) composed of one early and one late emerging individuals were isolated for 7 days in the same observation aquaria as described above. Following isolation, the dividing wall was carefully removed and fish were allowed to interact for 24 h after which social status was determined (see below).

A third set of experiments was performed during September 2009 to investigate if the behavioural differences detected in the first two sets of experiments were consistent over time. For this experiment, 16 early and 16 late emerging individuals were chosen from the holding tanks and isolated in 180 l glass aquarium. One late emerging individual died during the experiment and was removed from the analysis. Each aquarium was divided into 4 compartments by three opaque PVC walls so that 4 fish could be tested in the same aquarium. Fish were fed by hand for 10 days, and the time to resume feeding after isolation was recorded for each fish.

2.2. Resumption of feeding, metabolic rate and post stress cortisol concentrations

Since measurements of SMR were performed on two fish simultaneously (see below), one early and one late emerging fry were randomly selected from the holding tanks and isolated each day (mean body mass ± S.D. 1.6 ± 0.4 g for early and 1.4 ± 0.4 g for late emerging individuals, t = 1.4, d.f. = 20, p = 0.5). Following transport to the observation aquaria, fish were hand fed once a day ad libitum for 5 days. For each fish, resumption of feeding after isolation was quantified by measuring the number of pellets consumed and the latency to eat the first pellet in each day. If no pellets were consumed within 3 min, the latency was set to 180 s. At the end of each feeding observation, uneaten pellets were removed from the tank. The temperature during the tests was 12 ± 1 °C, which was similar to the water temperature in the holding tanks. After isolation for 5 days, fish were fasted for 48 h before the oxygen consumption (MO2) was measured. The recording was performed over an 18 hour period, using computerised intermittent flow-through respirometry, as described by Steffensen et al. [23]. Respirometers were constructed from glass cylinders sealed at both ends with a rubber stopper. A fibre optic oxygen sensor was inserted through one rubber stopper into the chamber, and oxygen saturation was registered every
Individuals were confined for 48 h before being subjected to a behavioural observation aquaria, where they were fed and allowed to swim above the mesh, while a miniature magnetic stirrer below ensured that the water temperature was approximately one third from the base. The test subject was placed horizontally in a chamber formed by a horizontal piece of plastic mesh was glued in place in each chamber. Each chamber corresponded to a peak of H3-cortisol (see further down), was scraped off into a borosilicate tube and extracted overnight with 5 ml ether. The next day the ether-silica was spun at 1300 rpm for 5 min and supernatant transferred into a new borosilicate tube and dried with nitrogen. The position of the cortisol spot was located by using H3-cortisol spiked samples with a mean recovery rate of 80%. Samples were redissolved in 500 μl of ethyl acetate and the concentration of cortisol was analysed by radioimmunoassay (RIA).

2.6. Statistical analysis

All values are presented as mean (±S.E.) unless otherwise stated. In the first set of experiments, repeated measures ANOVA followed by pairwise comparisons was used to investigate the differences between early and late emerging individuals in the number of pellets consumed and in latency to eat the first pellet during the 5 day isolation period. These observations were log transformed to attain normality. It was tested for the effects of number of days in isolation, emergence time and also the combined effect of days in isolation and time to emergence on the number of pellets consumed and on latency to eat the first pellet. Further on, a t-test was used to evaluate group differences in MO2. In addition, a two-way ANOVA followed by a Tukey HSD posthoc test was used to analyse effects of stress and emergence time on cortisol concentrations between the unstressed controls and confined individuals with an early or late time to emerge. Measures of post stress cortisol concentrations were log transformed to achieve normality. Pearson correlation coefficients and p-values were calculated to correlate the measurements of resumption of feeding, MO2, and post stress cortisol levels made on the same individuals.

In the second set of experiments, a chi test was used to investigate whether proportions of individuals becoming subordinate or dominant differed among individuals grouped as early or late emerging individuals. Resumption of feeding after transfer to isolation in the third set of experiments was analysed following a similar repeated measures ANOVA followed by pairwise comparisons as described for the first set of experiments. All the statistics were performed using the SAS 9.1 statistical software package.

3. Results

3.1. Resumption of feeding, metabolic rate and post stress cortisol concentrations

The latency to eat the first pellet decreased during the 5 days of isolation (F4, 80 = 10.9; p < 0.01). Furthermore, no differences were observed between early and late emerging fry for the latency to eat the first pellet (F1, 20 = 2.3; p = 0.1). The mean (±S.E.) latency to eat the first pellet for early emerged fry was 53.0 (±10.1) s and 87.3 (±19.0) s for late emerging fry. As well, there was a significant interaction between emergence time and isolation days (F4, 80 = 3.6; p < 0.01). For each day of isolation, no differences in the latency time between early and late emerging fry were observed on days 1, 2, and 3 (p > 0.05 for all tests). However, early emerged fry took less time to eat the first pellet at days 4 and 5 (p < 0.05 for all tests), compared to late emerging fry (Fig. 2a).

The number of pellets consumed increased during the days in isolation (F4, 80 = 20.9; p < 0.01). Moreover, the number of pellets eaten during 5 days of isolation did not differ between early and late emerging fry (F1, 20 = 2.2; p = 0.2). Early emerged fry consumed (79.2 ± 13.4) and late emerged fry consumed (50.1 ± 14.1). As well, there was a significant interaction between emergence time and days in isolation.
(F4,80 = 3.0; p = 0.02). Pairwise comparisons show no differences between early and late emerging fry on days 1, 2 and 3 (p > 0.05 for all tests). However, early emerging fry consumed more pellets on days 4 and 5 (p < 0.05 for both tests), compared to late emerging fry (Fig. 2b).

No differences in MO2 were found between individuals with different emergence times (t = −0.1, d.f. = 20, p = 0.9). Mean values (±S.E.) of MO2 were 164.6 ± 8.6 mg O2/kg/h for early and 165.4 ± 13.4 mg O2/kg/h for late emerging individuals.

Significant differences were found in post stress cortisol concentrations between the confined individuals and the unstressed controls (F1, 34 = 6.0; p = 0.02), where the unstressed controls had lower cortisol concentrations (Fig. 3). However, no differences in post stress cortisol concentrations were observed between early and late emerging individuals (F1, 34 = 0.1; p = 0.8; Fig. 3). Furthermore, the interaction effect between the stress condition (confined vs. unstressed) was independent of the emergence time (F1, 34 = 0.9; p = 0.4).

Although there was a significant negative correlation between the total number of pellets eaten and the average time to eat the first pellet, these two measurements were not correlated to MO2 or post stress cortisol levels (Table 1).

### 3.2. Social dominance

After 24 h of social interaction, 5 early and 4 late individuals had become socially dominant. This frequency difference was not significant (χ2 = 0.22; d.f. = 1; p = 1).

### 3.3. Consistency over time

The latency to eat the first pellet decreased with the days in isolation (F9, 261 = 39.0; p < 0.01). Irrespective of the days in isolation, no differences were observed between early and late emerging fry (F1, 29 = 2.3; p = 0.1). The mean (±S.E.) time to eat the first pellet for early emerging fry was 88.0 (± 7.9) s and for late emerging fry was 114.5 (± 14.5) s. A significant interaction was observed between emergence time and isolation days (F9, 261 = 2.7; p = 0.02). No differences were observed from days 1 to 7 (all tests, p > 0.05), but significant differences were present in days 8, 9 and 10 (p < 0.05 for all tests, Fig. 4).

In general, the number of pellets consumed increased during the days in isolation (F9, 261 = 30.8; p < 0.01). However, no differences were observed between early and late emerging fry in the number of pellets eaten (F1, 29 = 1.31; p > 0.05) and in the interaction between emergence time and isolation days (F9, 261 = 1.9; p = 0.1). The mean (±S.E.) number of pellets consumed by early emerging fry was 119.1 (± 18.3) and for late emerging fry it was 83.6 (± 25.4) pellets.

### 4. Discussion

In the present study, two-month-old fry of Atlantic salmon with an early emergence time from artificial spawning redds, consumed more pellets and took less time to eat the first pellet after 4 days in isolation, compared to late emerging fry. Furthermore, the latency to eat the first pellet was consistent over time, since it was also expressed five months after
emergence. Early emerging fry had a shorter latency time after 8 days in isolation, compared to late emerging fry. Thus, early emerging fry resumed feeding earlier after isolation in a new environment. Considering the fact that salmonid larva leave the reed in order to start exogenous feeding, this suggests that initial individual differences in the ability to exploit a new environment are still present five months after emergence. Early and late emerging fry, however, did not differ in MO₂, post stress cortisol concentrations or social status, traits that are known to differ in the proactive and reactive coping styles [2].

Time to resume feeding in a new environment has been used as a measure of boldness behaviour in salmonids [4,16,27]. Consequently, a faster time to resume feeding after isolation suggests a bolder behaviour in fry with an earlier emergence time in the present study. Time to resume feeding in a novel environment, however, involves potentially anxiogenic anorectic effects (novelty and transport) as well as traits related to hunger (metabolic rate and capacity to process food) [4]. In the present study, bolder behaviour was observed in individuals with a faster time to reach emergence and first feeding stage. This suggests that boldness could be related to larval development and yolk conversion ratio. Considering that salmonids which consume their yolk sac faster may also have higher metabolic rates (Vaz-Serrano, unpublished) and greater capacity to process food after switching to exogenous feeding, it cannot be excluded that the bolder behaviour in early emerging fry is related to hunger, rather than to overcome anxiogenic anorectic effects. However, differences in metabolic rate have been related to food processing time and energy conversion [28], and the absence of a difference in metabolic rate between early and late emerging fry indicates that boldness in this study is related to the time to overcome the anxiogenic effect of being transported and isolated.

In previous studies, resumption of feeding after isolation has been associated with locomotor activity during acute stress, social dominance and post stress cortisol concentrations in salmonid fish [25,27,29]. Furthermore, these traits have been proposed to be an indicator of stress coping styles [4]. Recently, the standard metabolic rate of an individual fish [4,13,15,36]. Some studies have demonstrated that hatchery reared fish are bolder and more aggressive than wild reared fish [13,15], suggesting that the selection in captive fish is directed towards a bolder and aggressive behavioural type. However, there are studies suggesting that behavioural variability increases as a consequence of a lack of selection pressure against maladaptive behaviours in captive animals [11,36]. Furthermore, this hypothesis predicts a high individual variability as well as a lack of relationship between the behavioural type and other traits, like growth and survival [11]. This was demonstrated in captive Arctic char (Salvelinus alpinus), where boldness to natural predator cues was not related to growth [37]. In the present study, no correlation was found between the resumption of feeding, metabolic rate or post stress cortisol levels (Table 1), indicating that a suit of correlated traits is not present in the studied fish. Therefore, these findings could be an indication that the selection in captive fish is relaxed rather than directional. However, this view is still debated since other studies have shown behavioural syndromes and stress coping styles in captive fish [13,38–40].

In summary, early emerging individuals of Atlantic salmon were bolder than late emerging individuals and this behavioural trait was consistent over time. This is coherent with studies in natural populations that suggest a relationship between stress coping styles and emergence time in salmonids. However, emergence time was not related to other behavioural and physiological characteristics of stress coping styles. This decoupling could occur in fish reared under hatchery conditions, where competition for resources is less severe than in nature, and further studies are needed to investigate if the differences in stress coping styles between early and late emerging individuals are present in wild populations of salmonids.

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