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 bolder 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 where inserted in 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 emerge 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 $\pm$ 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
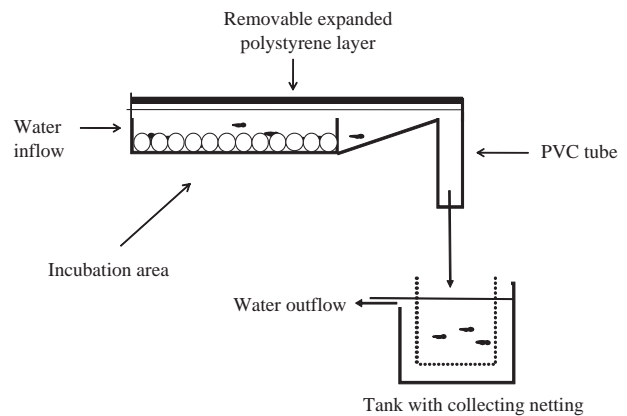
Fig. 1. Principal drawing of an incubator for sorting of Atlantic salmon larvae with respect to time to emerge from the artificial redds, in this case golf balls. Larvae were flushed downstream to a collecting tank when they emerged from the incubator.

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 20 l 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 aquaria. 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 $\pm$ 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 (MO_2) 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

second. A blunt needle with a diameter of an 18 G was inserted through each stopper, connected to a length of Tygon tubing for flushing the respirometer using a peristaltic pump (Ismatec SA, Switzerland). A horizontal piece of plastic mesh was glued in place in each chamber approximately one third from the base. The test subject was placed above the mesh, while a miniature magnetic stirrer below ensured complete mixing of the water volume in the respirometer. Measurements of MO_2 were performed in 16 minute intervals. Each interval consisted of a flush period lasting 240 s followed by a 120 s waiting period to reach steady-state conditions, and terminated by a 600 s measurement period during which the decline in water oxygen tension inside the respirometer was recorded using data acquisition software (AutoResp 4, Loligo Systems).

Following MO_2 measurements, individuals were returned to the behavioural observation aquaria, where they were fed and allowed to recover for 48 h before being subjected to a confinement stress test. Individuals were confined for 30 min in 50 ml containers with aerated water. Immediately after confinement, fish were exposed to a lethal dose of MS 222; carcasses were frozen on dry ice, and stored at −80 °C until further analysis of whole body cortisol concentrations. The unstressed controls were isolated and fed following the same protocol as described above, with the modification that fish were euthanized and frozen immediately after 5 days in isolation.

2.3. Social dominance

To investigate the relationship between time to emerge and social status, pairs of fish, consisting in one early and one late emerging fry, were isolated and hand fed for 7 days. Individuals were marked by upper or lower fin clipping. After the isolation period, the dividing wall separating the fish was lifted and the individuals were allowed to interact. This resulted in escalated fights in all pairs, and aggressive acts such as bites, chases and approaches were observed. This behaviour has been previously observed in salmonids during establishment of a hierarchy [24,25]. Social interactions were video recorded for 60 min. After the observation period, the aggression was unidirectional and dominant and subordinate fish were clearly distinguishable. The dominant–subordinate relationship was verified the next morning by behavioural observations during feeding. It was observed that the dominant fish swam around the aquarium and responded to the offered pellets, whereas the subordinate fish was immobile in a corner and did not respond to food.

2.4. Consistency over time

The above experiments, performed two months after emergence, indicated differences in the resumption of feeding after transfer to isolation between fish with an early and late time to emerge (see Results). Consequently, it was investigated if the observed difference was still present five months after emergence. The mean body mass (± S.D) of the fish in this test was 29.1 ± 4.8 g for early and 29.7 ± 5.4 g for late emerging fish (t = −0.3, d.f. = 29, p = 0.7). The water temperature during this experiment was 10 ± 1 °C, which was similar to the temperature in the holding tanks.

2.5. Cortisol assay

Frozen salmon carcasses were sent to the Department of Comparative Physiology, Uppsala University, Sweden. Each carcass was homogenised in a volume of PBS equivalent to the fish body mass. Five volumes of ethyl acetate were added to the fish homogenate, vortexed and centrifuged at 2000 rpm for 5 min. The supernatant was transferred to a borosilicate tube and dried under nitrogen. The pellet was resuspended in 100 μl ethyl acetate for fractionation by thin-layer chromatography (TLC) using a modification of the method described by Denver [26]. Briefly, the samples were loaded on TLC silica gel 60

glass plates (250 μm). The plates were then run in a mobile phase of toluene:cyclohexane (1:1) for approximately 1 h. Plates were air-dried and placed in a second mobile phase of chloroform:methanol (98:2) for approximately 1 h. Again the plates were air-dried and thereafter the first 2 cm of the loading line from each lane, which corresponded to the 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 MO_2. 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, MO_2, 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 ($F_{4, 80} = 10.9$; p<0.01). Furthermore, no differences were observed between early and late emerging fry for the latency to eat the first pellet ($F_{1, 20} = 2.3$; 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 ($F_{4, 80} = 3.6$; 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 ($F_{4, 80} = 20.9$; p<0.01). Moreover, the number of pellets eaten during 5 days of isolation did not differ between early and late emerging fry ($F_{1, 20} = 2.2$; 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
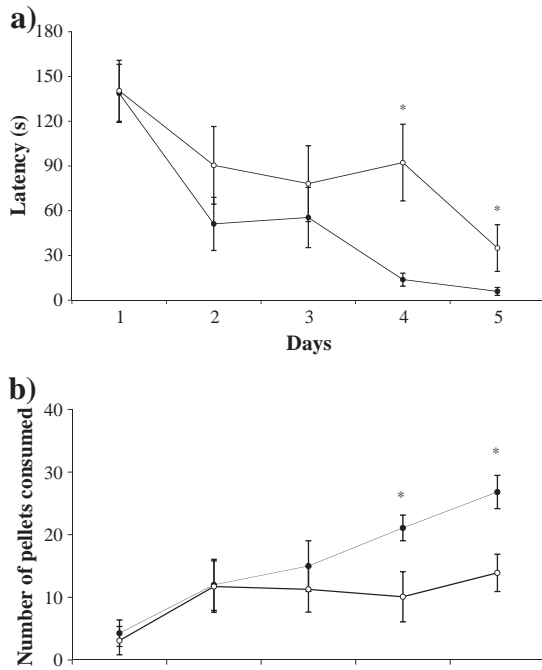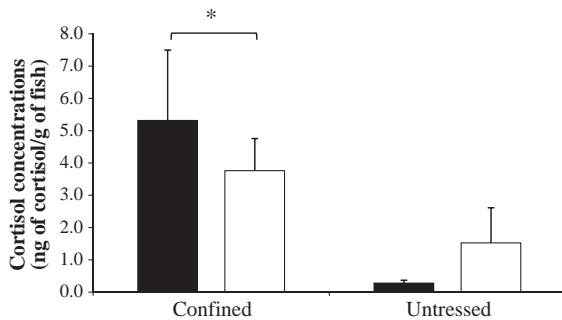
Fig. 2. The number of pellets consumed [a] and the latency (s) to eat the first pellet [b] during isolation for 5 days in groups of fish with an early (●) and a late (○) emergence time from artificial spawning redds, two months after emergence. N = 11 in each group.* Denotes significant difference between the early and late groups at the same time point (p < 0.05).

($F_{4,80} = 3.0$; $p = 0.02$). 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 MO_2 were found between individuals with different emergence times (t = − 0.1, d.f. = 20, p = 0.9). Mean values (± S.E.) of MO_2 were 164.6 ± 8.6 mg O_2/kg/h for early and 165.4 ± 13.4 mg O_2/kg/h for late emerging individuals.

Significant differences were found in post stress cortisol concentrations between the confined individuals and the unstressed controls ($F_{1, 34} = 6.0$; $p = 0.02$), 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 ($F_{1, 34} = 0.1$; $p = 0.8$; Fig. 3). Furthermore, the interaction effect between the stress condition (confined vs. unstressed) was independent of the emergence time ($F_{1, 34} = 0.9$; $p = 0.4$).

Although there was a significant negative correlation between the total number of pellets eaten and the average time to eat the first pellet,

Table 1
Pearson correlation values (R) between the total number of eaten pellets, the time to eat the first pellet, SMR (mg O_2/kg/h) and post stress cortisol concentrations (ng of cortisol/mg of fish). Values marked in bold are statistically significant (p < 0.05).

	Number of pellets	Time to eat the first pellet	SMR	Post stress cortisol concentrations
Number of pellets		**− 0.88**	0.04	0.05
Time to eat the first pellet			0.05	− 0.07
SMR				− 0.37
Post stress cortisol concentrations				

these two measurements were not correlated to MO_2 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 ($\chi^2 = 0.22$; d.f. = 1; p = 1).
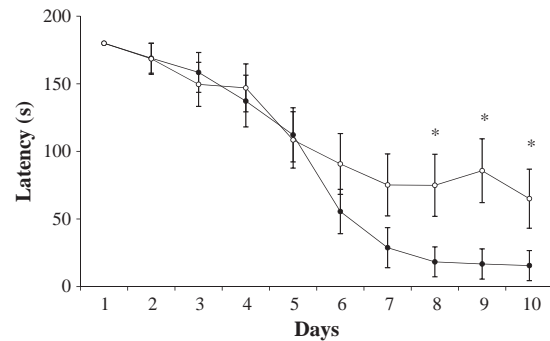
3.3. Consistency over time

The latency to eat the first pellet decreased with the days in isolation ($F_{9, 261} = 39.0$; p < 0.01). Irrespective of the days in isolation, no differences were observed between early and late emerging fry ($F_{1, 29} = 2.3$; 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 ($F_{9, 261} = 2.7$; 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 ($F_{9, 261} = 30.8$; p < 0.01). However, no differences were observed between early and late emerging fry in the number of pellets eaten ($F_{1, 29} = 1.31$; p > 0.05) and in the interaction between emergence time and isolation days ($F_{9, 261} = 1.9$; 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 time to emerge from artificial 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

Fig. 3. Post stress cortisol concentrations in groups of fish with an early (filled bars) and a late (open bars) time to emerge, in confined and unstressed conditions. N = 11 individuals in the confined groups and n = 8 in each unstressed control groups. * Denotes significant differences between the unstressed and stressed groups. No significant differences were found in the interaction between conditions and time to emerge (see text).

Fig. 4. Latency (s) to eat the first pellet in groups of fish with an early (●) and a late (○) emergence time from artificial spawning redds, during isolation for 10 days, five months after emergence. N = 16 in the group of early emerging time individuals and n = 15 in the group of late emerging time individuals. * Denotes significant difference between the early and late groups at the same time point (p < 0.05).

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 redd 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 MO_2, 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 transfer to 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 has also been suggested to be related to its stress coping style [19], where a higher metabolic rate reflects a more active, aggressive life style, such as the proactive stress coping style. In the present study, we could not detect any differences between early and late emerging individuals in post stress cortisol concentrations or metabolic rates. Furthermore, the social status of an individual was not related to the emergence time. This is in contrast to studies on natural populations of Atlantic salmon, where traits such as higher metabolic rate, probability to become socially dominant and willingness to take more risks in the presence of predators where observed in individuals that emerge early from the spawning nests [20,22], suggesting a relation between some traits of the stress coping and time to emergence.

The uncoupling between boldness and other stress coping styles traits in fry with an early or late emergence time observed in the present study might be a consequence of domestication. In wild populations of salmonids, a high selective pressure is present during emergence from the spawning nest and establishment of new territories [30–32]. The lack of feeding territories, the presence of predators, the variable environmental conditions, and/or aggression by a conspecific could result in a co-selection of suits of traits forming the stress coping styles. Furthermore, both genetic and environmental factors (e.g. social interactions and previous exposure to stress) contribute to extensive inter-individual variation in how a stressful experience affects behaviour and physiology [33–35]. Considering that a newly emerging individual may be naive to aggressive behaviour and social competition, the initial defence and establishment of territories could have profound effect on stress reactivity and behaviour. The bolder behaviour of the early emerging individuals may give them a competitive advantage, shaping their personality towards a more proactive stress coping style. In hatchery reared fish, on the other hand, this dramatic selection during emergence is absent.

According to several views, the selection pressure present in captive animals can be either directional or relaxed [11,13,15,36]. 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 copying 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.

Acknowledgements

References

[1] Wolf M, van Doorn GS, Leimar O, Weissing FJ. Life-history trade-offs favour the evolution of animal personalities. Nature 2007;447:581–4.

[2] Koolhaas JM, Korte SM, De Boer SF, Van Der Vegt BJ, Van Reenen CG, Hopster H, et al. Coping styles in animals: current status in behavior and stress-physiology. Neurosci Biobehav Rev 1999;23:925–35.

[3] Groothuis TGG, Carere C. Avian personalities: characterization and epigenesis. Neurosci Biobehav Rev 2005;29:137–50.

[4] Overli O, Sorensen C, Pulman KGT, Pottinger TG, Korzan W, Summers CH, et al. Evolutionary background for stress-coping styles: relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. Neurosci Biobehav Rev 2007;31:396–412.

[5] Overli O, Pottinger TG, Carrick TR, Overli E, Winberg S. Differences in behaviour between rainbow trout selected for high- and low-stress responsiveness. J Exp Biol 2002;205:391–5.

[6] Sih A, Bell A, Johnson JC. Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol Evol 2004;19:372–8.

[7] Coppens CM, de Boer SF, Koolhaas JM. Coping styles and behavioural flexibility: towards underlying mechanisms. Philos Trans R Soc Lond B Biol Sci 2010;365: 4021–8.

[8] Korte SM, Koolhaas JM, Wingfield JC, McEwen BS. The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. Neurosci Biobehav Rev 2005;29:3–38.

[9] Bell AM, Sih A. Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). Ecol Lett 2007;10:828–34.

[10] Brelin D, Petersson E, Dannewitz J, Dahl J, Winberg S. Frequency distribution of coping strategies in four populations of brown trout (*Salmo trutta*). Horm Behav 2008;53: 546–56.

[11] Conrad JL, Sih A. Behavioural type in newly emerged steelhead *Oncorhynchus mykiss* does not predict growth rate in a conventional hatchery rearing environment. J Fish Biol 2009;75:1410–26.

[12] Evans J, Boudreau K, Hyman J. Behavioural syndromes in urban and rural populations of song sparrows. Ethology 2010;116:588–95.

[13] Huntingford F, Adams C. Behavioural syndromes in farmed fish: implications for production and welfare. Behaviour 2005;142:1207–21.

[14] Bell AM, Stamps JA. Development of behavioural differences between individuals and populations of sticklebacks, *Gasterosteus aculeatus*. Anim Behav 2004;68: 1339–48.

[15] Huntingford FA. Implications of domestication and rearing conditions for the behaviour of cultivated fishes. J Fish Biol 2004;65:122–42.

[16] Ruiz-Gomez MD, Kittilsen S, Hoglund E, Huntingford FA, Sorensen C, Pottinger TG, et al. Behavioral plasticity in rainbow trout (*Oncorhynchus mykiss*) with divergent coping styles: when doves become hawks. Horm Behav 2008;54:534–8.

[17] Careau V, Thomas D, Humphries MM, Reale D. Energy metabolism and animal personality. Oikos 2008;117:641–53.

[18] Biro PA, Stamps JA. Are animal personality traits linked to life-history productivity? Trends Ecol Evol 2008;23:361–8.

[19] Huntingford FA, Andrew G, Mackenzie S, Morera D, Coyle SM, Pilarczyk M, et al. Coping strategies in a strongly schooling fish, the common carp *Cyprinus carpio*. J Fish Biol 2010;76:1576–91.

[20] Metcalfe NB, Thorpe JE. Early predictors of life-history events—the link between 1st feeding date, dominance and seaward migration in Atlantic Salmon, *Salmo salar* L. J Fish Biol 1992;41:93–9.

[21] Metcalfe NB, Taylor AC, Thorpe JE. Metabolic-rate, social-status and life-history strategies in Atlantic salmon. Anim Behav 1995;49:431–6.

[22] Brannas E. First access to territorial space and exposure to strong predation pressure—a conflict in early emerging Atlantic salmon (*Salmo salar* L) fry. Evol Ecol 1995;9:411–20.

[23] Steffensen JF, Johansen K, Bushnell PG. An automated swimming respirometer. Comp Biochem Physiol 1984;79:437–40.

[24] Overli O, Harris CA, Winberg S. Short-term effects of fights for social dominance and the establishment of dominant–subordinate relationships on brain monoamines and cortisol in rainbow trout. Brain Behav Evol 1999;54:263–75.

[25] Overli O, Korzan WJ, Hoglund E, Winberg S, Bollig H, Watt M, et al. Stress coping style predicts aggression and social dominance in rainbow trout. Horm Behav 2004;45:235–41.

[26] Denver RJ. Hormonal correlates of environmentally induced metamorphosis in the western spadefoot toad, *Scaphiopus hammondii*. Gen Comp Endocrinol 1998;110: 326–36.

[27] Overli O, Sorensen C, Nilsson GE. Behavioral indicators of stress-coping style in rainbow trout: do males and females react differently to novelty? Physiol Behav 2006;87:506–12.

[28] Millidine KJ, Armstrong JD, Metcalfe NB. Juvenile salmon with high standard metabolic rates have higher energy costs but can process meals faster. Proc R Soc Lond B Biol Sci 2009;276:2103–8.

[29] Kittilsen S, Ellis T, Schjolden J, Braastad BO, Overli O. Determining stress-responsiveness in family groups of Atlantic salmon (*Salmo salar*) using non-invasive measures. Aquaculture 2009;298:146–52.

[30] Bardonnet A, Gaudin P, Thorpe JE. Diel rhythm of emergence and of 1st displacement downstream in trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*) and grayling (*Thymallus thymallus*). J Fish Biol 1993;43:755–62.

[31] Milner NJ, Elliott JM, Armstrong JD, Gardiner R, Welton JS, Ladle M. The natural control of salmon and trout populations in streams. Fish Res 2003;62:111–25.

[32] Einum S, Sundt-Hansen L, Nislow KH. The partitioning of density-dependent dispersal, growth and survival throughout ontogeny in a highly fecund organism. Oikos 2006;113:489–96.

[33] Carere C, Eens M. Unravelling animal personalities: how and why individuals consistently differ. Behaviour 2005;142:1149–57.

[34] Frost AJ, Winrow-Giffen A, Ashley PJ, Sneddon LU. Plasticity in animal personality traits: does prior experience alter the degree of boldness? Proc R Soc Lond B Biol Sci 2007;274:333–9.

[35] Korzan WJ, Summers CH. Behavioral diversity and neurochemical plasticity: selection of stress coping strategies that define social status. Brain Behav Evol 2007;70:257–66.

[36] McPhee ME. Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. Biol Conserv 2004;115:71–7.

[37] Laakkonen MVM, Hirvonen H. Is boldness towards predators related to growth rate in naive captive-reared Arctic char (*Salvelinus alpinus*)? Can J Fish Aquat Sci 2007;64: 665–71.

[38] Brelin D, Petersson E, Winberg S. Divergent stress coping styles in juvenile brown trout (*Salmo trutta*). Ann NY Acad Sci 2005;1040:239–45.

[39] Silva PIM, Martins CIM, Engrola S, Marino G, Overli O, Conceicao LEC. Individual differences in cortisol levels and behaviour of Senegalese sole (*Solea senegalensis*) juveniles: evidence for coping styles. Appl Anim Behav Sci 2010;124:75–81.

[40] Van de Nieuwegiessen PG, Ramli NM, Knegtel B, Verreth JAJ, Schrama JW. Coping strategies in farmed African catfish *Clarias gariepinus*. Does it affect their welfare? J Fish Biol 2010;76:2486–501.