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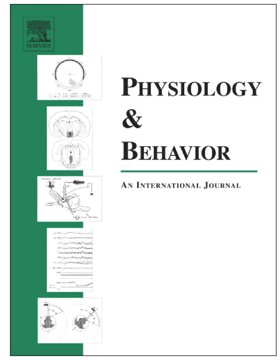

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Consistent individual competitive ability in rainbow trout as a proxy for coping style and its lack of correlation with cortisol responsiveness upon acute stress

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Abstract

For a given fish species, individuals are different in their ability to cope with stressors; each individual has its own set of physiological and behavioral responses to stress (stress-coping style). This individual diversity is of importance when considering the welfare of fish reared in aquaculture facilities. In this study with rainbow trout (*Oncorhynchus mykiss*) we investigated the link between the ability to compete for food of each individual (used as a proxy of dominance behavior/proactive stress-coping style) and its ability to cope with stress; we hypothesized that fish that are better competitors would be more robust against common aquaculture stressors. We screened 680 rainbow trout individuals for competition ability. This was done by submitting groups of 20 individuals to a 1-week competition trial where they were kept at low stocking density and were provided a restricted amount of food. A 15 % of the screened fish were selected as "winners" and another 15 % were selected as "losers", based on growth rates during the competition trials. Fish were re-tested in a second competition trial after several weeks, to assess for consistency of competitive ability. Winner and loser fish were individually exposed to confinement and their neuroendocrine stress response was evaluated (serotonergic activity in telencephalon and brain stem, plasma levels of cortisol, glucose and lactate). Furthermore, behavioral responses to confinement and net restraining tests were also investigated. The results showed good temporal consistency of competitive ability in the lapse of time of the experiments. Besides, competitive ability showed a positive association to fish activity during the net restraining tests. However, plasma stress marker data showed a lack of relevant differences between the acute stress responses of winner and loser fish, adding up to the body of evidence suggesting that stress responsiveness might not be consistently linked to SCS in vertebrates. This, together with the inability of winner fish to outperform loser fish in usual stocking density conditions, suggests that there is no clear welfare or performance benefits in selecting fish of a specific coping style for fish farming, at least in the domesticated trout population used in the current study.

KEYWORDS: individuality; competition; fish; aquaculture; welfare.

1. INTRODUCTION

Within a given species, fish, as other vertebrates, are known to display large inter-individual differences in the way they behave and in the way they cope with stress. The concept of stress coping styles (SCS) has been defined as "a coherent set of behavioral and physiological stress responses which is consistent over time and which is characteristic to a certain group of individuals" [1]. While there is a continuum in this set of behavioral and physiological responses to stress, two different extremes are usually considered, the so called proactive and reactive SCS. Proactive fish usually behave more actively when facing a stressor, are more aggressive, present a higher motivation for feeding and are more prone to take risks [2]. They also tend to become dominant in species that display social hierarchies [3,4]. Reactive fish tend to face stressors more passively, are more cautious and tend to be subordinate in social hierarchies [2]. From the physiological point of view, proactive fish have been shown to display higher sympathetic reactivity, but a lower responsiveness of the hypothalamus-pituitary-interrenal axis (HPI; responsible for the release of the stress hormone cortisol) upon stress than proactive fish [5–7], and some studies have also found higher metabolic rates in proactive than in reactive fish [8–10].

Fish individuality might be of key importance for aquaculture, both from the fish performance and fish welfare points of view, since individuals with different SCS are likely to be differentially affected by the stressors encountered during intensive production [11]. Some of the traits belonging to proactive fish, such as the higher feeding motivation [12,13] or the lower cortisol/HPI reactivity [7], seem to be more adequate for life in captivity. In fact, it has been shown that fish production systems may favor the selection of bolder, risk-taking fish [11,14], a behavioral trait usually associated to proactive fish [2]. In spite of this, it remains unclear whether selecting and farming proactive fish could be an adequate tool for improving fish performance and welfare in aquaculture. Most of the studies showing improved performance of proactive fish have been performed in conditions that strongly differ from those found in commercial farms [13,15–17], and several studies do not show performance differences between proactive and reactive fish [10,18]. Besides, the view of proactive fish as having a lower cortisol response to stress has been questioned and currently remains unclear, at least in salmonids [19–22].

In the present study, we selected rainbow trout juveniles based on the individual ability to compete for a limited amount of food in conditions of low stocking density. Competitiveness is believed to correspond well to the proactive-reactive duality and at the same time might be on itself a potential advantageous trait for aquaculture [2,15]. Our two main objectives were (i) to assess the temporal consistency of the individual competitive ability and (ii) to evaluate whether selecting the fish by their competitive ability could be a useful tool to improve fish welfare in an aquaculture setup. Our main hypothesis was that fish of consistently different competitive ability would have different behavioral and neuroendocrine responses to stress, which would highlight the value of this kind of behavioral selection as a potential tool to improve animal welfare in trout farms.

2. MATERIALS AND METHODS

2.1. Animals

Rainbow trout juveniles were obtained from a local trout farm (Lundby Fisk, Nibe, Denmark) and were transported to the facilities of the Technical University of Denmark. Upon arrival, the mass (SD) of the fish was 85.9 g (17.1 g). The fish were allocated in 600 L tanks at a density of 20 Kg m^{-3} (approx. 140 fish per tank). The tanks were part of a recirculating system with the water temperature kept at 15 °C. The water quality was daily monitored for NO_3^-, NO_2^-, NH_3/NH_4^+, pH and O_2 saturation, the latter being kept at all times above 80 %. Fish were daily fed a commercial trout feed (EFICO E 920, Biomar, Brande, Denmark; 1 % body mass day^{-1}). After a 4-week acclimation period, the procedures for fish selection started as described below. The use of fish in this study complied with Danish and EU legislation (Directive 2010/63/EU) on animal experimentation.

2.2. Fish selection

Fish were selected according to their ability to compete for food in conditions of low stocking density and reduced food availability [20], as follows. Fish were weighed under anesthesia (isoeugenol - Aqui-S ®, 180 mg L^{-1}) and 20 size-matched fish (body mass coefficient of variation below 10%) were allocated together in a 600 L circular tank. The resulting stocking density was always lower than 5 kg m^{-3}. Starting on the day after allocation, an amount of feed corresponding to 0.5 % of the biomass in the tank was administered daily along a 12 h period by using mechanical belt feeders. After seven days, the fish were re-weighed under anesthesia (isoeugenol - Aqui-S ®, 180 mg L^{-1}) to calculate the specific growth rate (SGR) for each individual, using the formula:

$$SGR = 100 * (LN(\text{final mass}) - LN(\text{initial mass}))/\text{time (days)}.$$

Individual fish were identified based on the pattern of melanin spots on the head [23]. The three individuals with higher SGR were selected as winners (W), and the three with the lower SGR were selected as losers (L) of the competitive event. In some cases there was a tie in the SGR value for the individual ranked 3rd; 4 individuals were selected as W or L in those cases. All the fish with intermediate SGRs were not used in the subsequent experiments. This competition setup was repeated a total of 34 times (using 4-6 experimental tanks at a time) to finally select a total of 115 W and 110 L fish. The selection process was completed in 8 weeks (Figure 1). The mass (SD) of the selected fish varied from 102.9 g (13.8 g), at the beginning of the selection process, to 135.1 g (24.9 g), in the last competition round.

W and L fish were reared in separate tanks (2 tanks were used for each of the groups, 4 tanks in total), in the same conditions described above in section 2.1. However, during the selection process, a slightly increased feed ration was given to the fish selected as L, to compensate for the unbalanced growth between the W and L fish induced by the 7-day competition. One week after the last selection round, the feed ration was stablished at 0.8 % fish mass day^{-1} for both groups.

The average individual mass of L and W fish was periodically evaluated after selection to monitor their growth (Figure 1). A number of fish from each group (n = 10-26) were netted at random, anesthetized (isoeugenol - Aqui-S ®, 180 mg L^{-1}) and individually weighed. This was done 2 weeks after the last selection round and also when selecting the L and W fish for the second competition trials (see below).

2.3. Experimental design

Experiment 1: Acute confinement stress challenge

Four weeks after the last selection round L and W fish were exposed to an acute stress challenge, consisting in a confinement stress protocol. Fish from both groups (n = 12 per group) were netted from their home tanks and allocated individually in 50 L tanks containing 10 L of water and air supply. Tanks were closed with a plastic lid, to prevent the fish being further altered by external stimuli. After 40 min, the fish were recaptured and anesthetized by immersion in (isoeugenol - Aqui-S ®, 180 mg L^{-1}). A 100 µL blood sample was collected from the caudal vessels with heparin-rinsed syringes, and the fish were then delivered back to their home tanks. Control fish (n = 10 per group) were collected from the L and W home tanks and were directly anesthetized for blood collection. Blood samples were centrifuged (5000 g, 10 min, 4°C) and plasma collected and stored at -80 °C until analysis of cortisol, glucose and lactate. The average mass (SD) of the fish in this experiment was 177.6 g (44.5 g).

Experiment 2: Second competitive trial followed by confinement and net restraining tests.

A total of 85 W and 85 L fish were submitted to a second competition test (Figure 1) in order to assess for consistency of the competitive behavior. The average mass (SD) of the fish used in this second competitive trial was 206.9 g (45.3 g). A set of 5 L and 5 W fish were weighed under anesthesia (isoeugenol - Aqui-S ®, 180 mg L^{-1}) and allocated together in a 600 L circular tank. The competitive trial was carried out as explained before, but using only 10 individuals in the tank instead of 20. The number of fish per tank in this second competition test was reduced to keep stocking density comparable to that of the selection process (lower than 5 kg m^{-3}). The competitive setup was repeated a total of 17 times (using 4-6 experimental tanks at a time). The trial was completed in 7 weeks (Figure 1). At the end of each competitive trial, the fish were captured and directly submitted to an acute confinement stress protocol, as explained above. In this case, the behavior of each individual was video-monitored through a hole in the lid of the tank, as described below. In 12 out of the 17 replicated competitions, the fish were sampled for blood as explained for experiment 1, and were then sacrificed by an anesthetic overdose (isoeugenol - Aqui-S ®, 360 mg L^{-1}). Immediately after, the brain of each individual was dissected out. The telencephalon and the brain stem were stored at -80 °C for the subsequent analysis of serotonergic metabolites. In these trials, 4 out of 10 fish in the tank were used as controls, and were not submitted to the stress protocols. Sampling of the control fish for plasma and brain tissues was done as described for stressed fish.

In the rest of the cases (5 out of 17 replicated competitions), the fish were, after the confinement protocol, further exposed to a net restraining test (adapted from Castanheira et al., 2016, 2013), as follows. From the confinement tanks, the fish were directly poured to a dip net (left in the air) and the behavior of the fish was video-monitored for 1 min. After that the fish were immediately killed by anesthetic overdose (isoeugenol - Aqui-S ®, 360 mg L^{-1}).

At the end of the performed tests, the mass of each individual was recorded, either in fish sampled for blood and brain or in the fish submitted to the net restraining tests. The SGR of each individual during the second competition trial was calculated, and in each case, the three individuals showing the lower and higher SGR were categorized as the 2nd competition losers (L') and the 2nd competition winners (W'), respectively.

2.4. Behavioral analysis

Confinement

In experiment 2 the behavior of each individual during the confinement period was video-recorded for 5 min. Videos were always taken within the period between 10 min and 25 min after the start of the confinement. A zenithal view was obtained from a hole in the lid of the confinement tank. During video analysis, different parameters were obtained from the 5-min videos. Those included the opercular beating rate (beats min^{-1}), percentage of time spent moving, total distance covered (m) and average speed ($m\ s^{-1}$).

Net restraining test

From the 1-min videos the following parameters were quantified: Duration of an initial immobile phase, number of “weak movements” (defined as movements which did not result in flips or displacement), number of “strong movements” (defined as movements resulting in jumps and/or displacement), total number of movements, longer string of strong movements (with no lag above 1 s between movements), and number of pauses (periods of immobility longer than 3 s).

All parameters were quantified with the help of behavior-coding software (Solomon Coder ©, v. 17.03.22, www.solomoncoder.com).

2.5. Biochemical analysis

Plasma cortisol concentrations were measured using a commercial ELISA kit (ref. 402710, Neogen Europe, Ayrshire, UK), following the manufacturer’s instructions. Plasma glucose and lactate were analyzed with colorimetric kits from Sigma (ref. MAK013 and MAK064, respectively; St Louis, MO, USA).

Concentration of serotonin (5-hydroxytryptamine, 5-HT) and its main oxidative metabolite, 5-hydroxyindoleacetic acid (5-HIAA) were quantified by means of HPLC with electrochemical detection, as previously described [20].

2.6. Statistical analysis

All statistical analyses were performed with SigmaPlot version 12.5 (Systat Software Inc., San Jose, CA, USA). In the first and second competitive trials, mass before and after competition and individual SGR of L and W fish were averaged per competitive trial unit and then compared using paired t-tests (or Wilcoxon signed-rank test, when assumption of normality was not fulfilled). The levels of the plasma stress markers in experiment 1 (cortisol, glucose, lactate) were compared by two-way ANOVA with competitive group (L versus W) and treatment (control versus stress) as independent variables, followed by post hoc comparisons with the Holm-Sidak test. To evaluate the growth of L and W fish, their average mass was compared over time using two-way ANOVA with time and competitive ability as independent variables. To determine the consistency of the competitive behavior, the relative contribution of L and W fish to L' and W' groups was analyzed by a Pearson's chi-squared test. The plasma and brain stress markers in experiment 2 were compared using two-way ANOVA with competition ability and stress as independent variables, followed by post hoc comparisons with the Holm-Sidak test. Behavior-related variables in experiment 2 were compared using Student's t-tests (or Mann-Whitney U tests, when normality or equality of variance assumptions were not fulfilled). Those behavioral parameters were assessed in two ways. First, data were compared between the L' and W' (i.e. the losers and winners of the 2nd competition). Secondly, the data were also compared between the original selection groups, W and L. Statistical significance was set at $p < 0.05$.

3. RESULTS

Selection of L and W fish

Figure 2 shows the individual mass before and after the competition during the selection process, as well as the corresponding SGRs, for the fish categorized and selected as L and W. There were no differences in the initial mass. After the 7-day competition events, the mass of the W fish was on average 20 % higher than that of L fish.

Stress markers in W and L fish

In the first stress experiment, the confinement protocol induced a clear increase in cortisol, glucose and lactate levels (Figure 3 and Table 1). A significant main effect of the competition group ($p = 0.044$) was found in glucose levels, which were 12 % higher in the L than in the W fish, independently of the confinement stress, but no post-hoc differences were found between L and W fish within any of the specific treatment groups. No interaction was found between the stress response of any of the markers and the competition ability of the fish.

Growth during the interval between competitive tests

The average mass of L and W fish after selection and before the second competitive trial is shown in Figure 4. Fish grew over time ($F_{4,171}$ = 21.8, $p < 0.001$) but no performance differences between L and W fish ($F_{1,171} = 0.258$, $p = 0.612$) and no interactions between time and competition group ($F_{4,171} = 0.036$, $p = 0.997$) were found.

Consistency of the competitive behavior

Individuals categorized as L or W in the first competitive trial had an enhanced chance to become L' or W' in the second trial, respectively (chi-square = 20.8, $p < 0.001$). Among the 36 selected W' individuals, 27 were from the W group. Among the 36 L' individuals, 30 belonged to the L group (Figure 5A). Also, fish originally categorized as L showed during the second competition trial a lower SGR (Mann-Whitney U-test, $U = 872.0$, $p < 0.001$) than fish originally categorized as W (Figure 5B).

Plasma stress markers after confinement tests immediately after the 2nd competition

Figure 6 shows the response of cortisol, glucose and lactate to confinement in the fish found to be the losers and winners of the second competition event (L' and W', respectively). The n in this trial was variable, since the fish were randomly allocated to control or stressed groups before knowing the result of the competition (which was evaluated later based on the SGR of each individual). In all three parameters there was a clear increase as a result of the stress protocol (Figure 6 and Table 1). In the case of lactate, the two-way ANOVA analysis found an interaction between the effect of stress and the competitive group (Table 1). The simple main effects in this case showed that lactate levels were higher in W' than in L' fish within the controls but not within stressed fish. Besides, stressed fish showed higher lactate levels than control fish, for both L' and W' fish. The stress protocol also induced increases of 5-HIAA and 5-HIAA/5-HT in both the telencephalon and the brain stem (Figures 7 and 8, Table 1). In the case of the brain stem, an effect of the competition ability was found in both 5-HIAA and 5-HIAA/5-HT, with higher post-stress levels in the L' than in the W' group (Figure 8, Table 1).

Behavior in confinement tests

The inter-individual variation in the parameters assessed in this test was large, with the exception of the opercular beat rate (Table 2). A relevant percentage of the tested fish, similar for both competition groups, remained inactive during the test. No effects were found between L' and W' fish in any of the behavioral parameters assessed in these tests. Similarly, when taking into account the original L and W categories, no changes were observed (Table 2).

Behavior in net restraining tests

After falling in the net and moving once or twice, most of the fish had an initial pause, whose duration was quantified. After this pause, fish started to move by beating the tail in series/strings. No effects were observed between L' and W' fish in any of the behavioral parameters assessed in this test (Table 2). However, the results showed an effect of the original competition group in the number of strong movements and in the jumps string, which were lower in L than in W fish (Table 2).

4. DISCUSSION

In order to evaluate the relevance of fish individuality in terms of welfare, it is necessary at first to characterize the behavioral type or SCS of the different individuals in the population to be studied. Different methods of behavioral screening have been used in fish, based on individual, pairwise or group behavioral tests (reviewed in Castanheira et al., 2017). Each kind of method has inherent advantages and disadvantages, that might be different depending on the objective or the fish species of study. While individual and pairwise tests allow for a precise assessment of fish behavior, and perhaps a better characterization of individual SCS, they are impractical when a rapid screening of high numbers of fish is needed. Depending on the aim of the study, they might also be inadequate when working with shoaling species. Different group-based tests have been recently used, such as group risk-taking tests [18,26], or hypoxia-avoidance tests [27–29], which are expected to characterize the fish based on their boldness. Those tests allow for the rapid screening of high numbers of fish, based on the latency for each individual to move from one to another zone of the experimental setup. Another advantage of these methods relates to the fact that the shoaling behavior in social species can be respected, which may help to avoid isolation-related stress associated to individual/paired tests. However, the potential interference of random factors on the screening is relatively high and therefore, the precision in the estimation of the individual SCS is expected to be lower than in individual or pairwise tests. Furthermore, the behavior of the fish in those tests could be difficult to interpret: In hypoxia avoidance tests, fish are usually given the chance to choose between a protected area in increasingly hypoxic water and a bright, exposed area containing normoxic water. Fish are then classified according to the latency to abandon the protected, hypoxic area. When the latency is higher, this can be interpreted as a tendency to avoid risks (a reactive-like trait) or as a higher resilience to tolerate environmental stressors or as a result of lower behavioral flexibility (proactive-like traits) [28,29]. In the current study, we used an intermediate approach between individual/paired and high throughput group tests for behavioral screening, trying to find a compromise between the advantages and disadvantages of both kinds of methods. We performed competitive trials with a reduced number of fish, promoting a competitive environment by reducing the stocking density and the availability of food. In these conditions, proactive fish, more aggressive/dominant and with a higher feeding motivation, are expected to be able to dominate space and food over fish of more reactive SCS [30]. Therefore, fish screened as W and L, showing extreme SGR in these competitions, were assumed to be fish of more proactive and more reactive SCS, respectively. These kind of competitive tests are expected to provide precise information about the fish competitive ability/SCS, since SGR values do not merely represent a punctual behavioral response, but are integrating the fish food intake during the 7-day trial period. At the same time, they allow for a faster SCS characterization than individual or pairwise behavioral tests.

While many studies evaluated behavioral and physiological differences between fish of different coping style, not much is known about the true determinants of individual differences. Genetic factors are believed to be involved in fish coping behavior [18,31–33] but environmental factors and fish experience have been shown to induce behavioral changes in fish [34]. In this regard, fish are known to have a high level of behavioral plasticity. For example, boldness and other traits such as metabolic rates, performance or stress responsiveness, which are often used for characterizing fish SCS in behavioral tests, are known to be affected by the social environment and other environmental factors [25,35–39]. On the other hand, also aggression and dominance behavior depend on the social environment, and it is known that fighting for dominance in salmonids and other fish is usually reduced when the stocking density is relatively high [40–43]. Given that plasticity, it is perhaps understandable that fish behavioral traits display diverse levels of consistency across time and context. However, available information about the consistency of behavioral traits associated to SCS in fish is very limited. While some studies show relatively good temporal and across-context consistency in the responses of fish to behavioral tests [3,24], other studies point to a lack of consistency, particularly when the consistency is tested across long periods or when the conditions of the fish change between tests [25,44]. In general, the consistency seems to vary depending on the species, the trait evaluated, the test used and the environmental conditions previously or during the tests. To which extent the lack of consistency is the result of a true change in fish personality or just due to punctual changes in the specific response evaluated in the used tests is difficult to know. In the current study, the time passed between the 1st and 2nd competition trial was different for each individual but ranged between 4 and 12 weeks. The level of consistency was high, since 75 % of the winners and 83 % of the losers of the 2nd competition had been previously characterized as W and L in the first competition, respectively. Also, W fish displayed a higher SGR than L fish during the second competition, suggesting that the competitive ability was conserved to a large extent. Interestingly, this high level of consistency in competitive behavior was found even when the W and L fish were transferred, after the first competition, to new tanks were the social environment was changed with respect to their previous conditions: fish were allocated together with fish of similar competitive ability and the stocking density of the W and L tanks was sequentially increased as long as the first competitions proceeded. As commented above, social context can have strong effects on fish behavioral traits, but in this case the consistency of fish behavior was still high. Furthermore, during the interval between competition trials, no relevant differences were found between W and L fish in terms of growth, which is probably just reflecting the lack of competition in conditions of high density. These data suggest that individuals that are better competitors have no real advantage over other individuals when the rearing conditions (i.e. high stocking density) do not promote a competitive environment, which is usually the case in the fish farming industry, where the stocking density is usually kept relatively high and fish have access to adequate amounts of feed. Similar results were found by Pottinger (2006), after rearing trout of different coping style (selected for divergent cortisol response to stress) separately or in co-culture. When reared separately at high density both groups showed similar performance, but the proactive group outperformed the reactive group when fish from both groups were co-reared at low density. In that study, the contextual differences found for growth were attributed to the differential behavior of both groups when facing different kind of competitors, rather than to the differential stocking density. Our data however do not support that explanation, since the fish selected as W and L were co-reared at high density and presented no differences in size before the start of our experiments, as shown in Figure 2.

After the first competition trial and the characterization of the selected fish as L or W, an acute stress challenge trial was performed in order to see whether the fish from both groups differed in their physiological response to stress. Individuals of each group were exposed to confinement by being individually transferred to a new tank containing a reduced amount of water. Confinement tests have been often used as a way to characterize fish behavioral and physiological responses to acute stress [46–49]. In this first stress challenge trial the plasma levels of cortisol, glucose and lactate were used as markers of the stress experienced by the fish [50,51]. While confinement induced a clear increase of all three of them, no differences were found between L and W fish (beyond slightly higher glucose levels in L fish, independent of the stress condition). These results pointed to a lack of correlation between the competitive behavior of the fish and the activity of the HPI axis, responsible for the production and release of cortisol under stress. This is in disagreement with a previous study with common carp [15] that demonstrated a negative association between fish competitive ability and the HPI axis sensitivity to acute stress in that species. However, the first stress-challenge test in the current study was performed several weeks after the characterization of the W and L groups and, as discussed above, the known high behavioral plasticity of fish could have affected the individual differences among individuals during that time. That happened for example in a study of Thomson and cols. (2016) [52], where a correlation between boldness and post-stress cortisol response was lost after submitting the fish to a different social environment. Therefore, we repeated the confinement challenge test immediately after the second competition trial to be able to evaluate the stress response of each individual along with their competitive ability with no time gap in between. The response of cortisol, glucose and lactate plasma levels to confinement was very similar to that observed in the first stress-challenge trial. All three markers were increased after stress, but no large differences in the response were found between L' and W'. In the control group plasma lactate levels were higher for W' than for L' fish. The reason behind that difference is not known but, since lactate levels usually increase along with muscle activity. It could perhaps be related to a higher activity of the W' fish in the tank during the competition event in order to keep control of the feeding spot. Altogether, the results of both stress challenge trials strongly suggest that the HPI response to acute stress was similar between fish of different competitive ability, in this trout population. This is in support of the increased amount of evidence showing a lack of consistency in the relationship between HPI responsiveness and coping style in fish and other vertebrates [19,21,22,53,54]. This differs from the usual view of reactive fish as of having a greater responsiveness of cortisol to acute stress than proactive fish [2,55]. Even when a genetic link might exist between behavior and the physiological stress responses, it has been suggested that the plastic nature of vertebrate behavior and the different subjective environmental conditions can easily uncouple both aspects of coping styles [52]. In the same direction, it has been also suggested that the decoupling between HPI responsiveness and behavioral and life history traits usually associated to stress coping styles might be favored by the process of domestication [20,53,56].

The release of cortisol and other stress hormones (i.e. catecholamines) into circulation, and the subsequent increase of energy metabolites such as plasma and lactate are just the final steps of a neuroendocrine cascade controlled by the two main axes driving the stress responses: the HPI axis and the brain–sympathetic–chromaffin (BSC) axis [50]. In the current study, we wanted to get some information to investigate the stress response of the fish at the level of the CNS. The serotonergic system is believed to be involved in the organization of the integrated stress response in vertebrates [51,57–59], and the activity of serotonergic neurons has been show to increase consistently upon stress exposure in fish [51,56]. The ratio between 5-HIAA and serotonin is used as an indirect estimator of the serotonergic activity, since 5-HIAA is usually produced after degradation of part of the 5-HT released at the synaptic terminal [59,60]. We chose to analyze the serotonergic activity in the telencephalon and in the brain stem after the second stress challenge trial. The fish telencephalon is believed to host structures involved in the emotional reactivity and high-level processing in the fish brain [29], and has been consistently demonstrated to show stress-induced effects in its serotonergic outcome in fish. The brain stem contains a large amount of serotonergic neuron bodies (the raphe serotonergic populations) [61] and has been shown to be involved in the differences between fish of different coping style [62]. As expected, confinement stress induced an increase in 5-HIAA and in the serotonergic ratio in the telencephalon and the brain stem in both L' and W'. We found no differences in the serotonergic response between L' and W' in the telencephalon but the post stress levels of 5-HIAA and the serotonergic ratio were higher in the brain stem of L' fish when compared to W'. This brain region-specific difference is remarkable and is in agreement with a previous study that showed that rainbow trout with higher feeding score presented a lower brain stem serotonergic response to 1 week of isolation [62]. The cause or the functional relevance of this difference are not known, but had no effects in the response of the HPI axis to the stressor as commented before.

Fish individuals classified as W during the first competitive trial had a better ability to perform strong movements in the net restraining test than L fish, since they displayed longer strings and a higher total number of strong movements. A similar trend was observed between W' and L' fish but was not found to be statistically significant, perhaps because of the reduced number of samples. The positive association between competitive ability and the fish activity during the net restraining tests demonstrates behavioral consistency across contexts for the W and L fish and is in agreement with other studies showing that proactive fish tend to display higher activity levels in net restraining tests [24,63]. Potentially, the higher activity of W fish could be related to a higher availability of energy metabolites, since W fish were eating more than L fish during the 7-day competition period just before the net restraining tests. However, the plasma glucose levels of both W and L fish were similar after the competition and therefore, the higher activity of the W fish during the net restraining test is likely the result of a higher motivation for an active escape response (more proactive behavior) of those fish.

In conclusion, the individual ability of rainbow trout to compete for limited food in conditions of low stocking density was consistent across a time span of up to 12 weeks. The competitive ability was associated with some behavioral traits observed during net restraining tests, which demonstrates also some consistency across context. However, the presented results suggest that the HPI responsiveness to acute stress was not linked to the animal coping style, and therefore both competitive groups, winners or losers seemed to be equally affected by the stressors applied. Furthermore, when these fish of consistently different behavior were reared (mixed or segregated) in conditions that did not promote competition (larger densities, larger feed rations) no differences in growth were observed associated to their competitive ability, showing that performance differences between fish of different coping style depend on rearing conditions. Most usually, fish conditions in salmonid fish farms do not promote extensive competition for resources, since stocking densities are relatively high and feed is provided at a ration directed to maximize growth rates. Therefore, the observed results refuted our initial hypothesis and suggested that there are no clear welfare or performance benefits in segregating fish of different coping style, at least in the domesticated trout population used in the current study and in relation to intensive fish farming. Still, it is important to highlight that, as commented above, some individuals are consistently better competitors than others, and that this might have relevant welfare and growth performance implications when farming salmonids at low stocking densities, as in organic aquaculture farms.

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REFERENCES

[1] J.M. Koolhaas, S.M. Korte, S.F. De Boer, B.J. Van Der Vegt, C.G. Van Reenen, H. Hopster, I.C. De Jong, M.A.W. Ruis, H.J. Blokhuis, Coping styles in animals: Current status in behavior and stress- physiology, Neurosci. Biobehav. Rev. 23 (1999) 925–935. doi:10.1016/S0149-7634(99)00026-3.

[2] M.F. Castanheira, L.E.C. Conceição, S. Millot, S. Rey, M.L. Bégout, B. Damsgård, T. Kristiansen, E. Höglund, Ø. Øverli, C.I.M. Martins, Coping styles in farmed fish: consequences for aquaculture, Rev. Aquac. (2017). doi:10.1111/raq.12100.

[3] J. Schjolden, A. Stoskhus, S. Winberg, Does Individual Variation in Stress Responses and Agonistic Behavior Reflect Divergent Stress Coping Strategies in Juvenile Rainbow Trout ?, Physiol. Biochem. Zool. 78 (2005) 715–723. doi:10.1086/432153.

[4] S.J. Dahlbom, D. Lagman, K. Lundstedt-Enkel, L.F. Sundström, S. Winberg, Boldness predicts social status in zebrafish (Danio rerio), PLoS One. (2011). doi:10.1371/journal.pone.0023565.

[5] D. Brelin, E. Petersson, S. Winberg, Divergent stress coping styles in juvenile brown trout (Salmo trutta), in: Ann. N. Y. Acad. Sci., 2005. doi:10.1196/annals.1327.033.

[6] T. Backström, E. Brännäs, J. Nilsson, C. Magnhagen, Behaviour, physiology and carotenoid pigmentation in Arctic charr Salvelinus alpinus, J. Fish Biol. (2014). doi:10.1111/jfb.12240.

[7] S. Winberg, E. Höglund, Ø. Øverli, Variation in the Neuroendocrine Stress Response, in: Fish Physiol., 2016: pp. 35–74. doi:10.1016/B978-0-12-802728-8.00002-3.

[8] C.I.M. Martins, M.F. Castanheira, S. Engrola, B. Costas, L.E.C. Conceição, Individual differences in metabolism predict coping styles in fish, Appl. Anim. Behav. Sci. (2011). doi:10.1016/j.applanim.2010.12.007.

[9] M. Yuan, Y. Chen, Y. Huang, W. Lu, Behavioral and metabolic phenotype indicate personality in zebrafish (Danio rerio), Front. Physiol. (2018). doi:10.3389/fphys.2018.00653.

[10] P.V. Skov, K.J. de Jesus Gregersen, M. Gesto, A. Jokumsen, Proactive coping style in early emerging rainbow trout carries a metabolic cost with no apparent return, Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 231 (2019) 104–110. doi:10.1016/j.cbpa.2019.01.016.

[11] F.A. Huntingford, S. Kadri, Welfare and Fish, in: Fish Welf., 2008. doi:10.1002/9780470697610.ch2.

[12] T.S. Kristiansen, A. Fernö, Individual behaviour and growth of halibut (Hippoglossus hippoglossus L.) fed sinking and floating feed: Evidence of different coping styles, Appl. Anim. Behav. Sci. (2007). doi:10.1016/j.applanim.2006.09.007.

[13] J. Mas-Muñoz, H. Komen, O. Schneider, S.W. Visch, J.W. Schrama, Feeding behaviour, swimming activity and boldness explain variation in feed intake and growth of sole (Solea solea) reared in captivity, PLoS One. (2011). doi:10.1371/journal.pone.0021393.

[14] F.A. Huntingford, Implications of domestication and rearing conditions for the behaviour of cultivated fishes, J. Fish Biol. 65 (2004) 122–142. doi:10.1111/j.1095-8649.2004.00562.x.

[15] F.A. Huntingford, G. Andrew, S. Mackenzie, D. Morera, S.M. Coyle, M. Pilarczyk, S. Kadri, Coping strategies in a strongly schooling fish, the common carp Cyprinus carpio, J. Fish Biol. (2010). doi:10.1111/j.1095-8649.2010.02582.x.

[16] C.I.M. Martins, J.W. Schrama, J.A.J. Verreth, The consistency of individual differences in growth, feed efficiency and feeding behaviour in African catfish Clarias gariepinus (Burchell 1822) housed individually, Aquac. Res. (2005). doi:10.1111/j.1365-2109.2005.01372.x.

[17] C.I.M. Martins, B. Hillen, J.W. Schrama, J.A.J. Verreth, A brief note on the relationship between residual feed intake and aggression behaviour in juveniles of African catfish Clarias gariepinus, Appl. Anim. Behav. Sci. (2008). doi:10.1016/j.applanim.2007.06.017.

[18] S. Ferrari, K. Horri, F. Allal, A. Vergnet, D. Benhaim, M. Vandeputte, B. Chatain, M.-L. Bégout, Heritability of Boldness and Hypoxia Avoidance in European Seabass, Dicentrarchus labrax, PLoS One. 11 (2016) e0168506. doi:10.1371/journal.pone.0168506.

[19] P.-O. Thornqvist, E. Hoglund, S. Winberg, Natural selection constrains personality and brain gene expression differences in Atlantic salmon (Salmo salar), J. Exp. Biol. 218 (2015) 1077–1083. doi:10.1242/jeb.114314.

[20] M. Gesto, P. V. Skov, A. Jokumsen, Emergence time and skin melanin spot patterns do not correlate with growth performance, social competitive ability or stress response in farmed rainbow trout, Front. Neurosci. 11 (2017). doi:10.3389/fnins.2017.00319.

[21] J.M. Koolhaas, S.F. de Boer, C.M. Coppens, B. Buwalda, Neuroendocrinology of coping styles: Towards understanding the biology of individual variation, Front. Neuroendocrinol. 31 (2010) 307–321. doi:10.1016/j.yfrne.2010.04.001.

[22] J. Qu, Q.E. Fletcher, D. Réale, W. Li, Y. Zhang, Independence between coping style and stress reactivity in plateau pika, Physiol. Behav. (2018). doi:10.1016/j.physbeh.2018.09.007.

[23] J.E. Merz, P. Skvorc, S.M. Sogard, C. Watry, S.M. Blankenship, E.E. Van Nieuwenhuyse, Onset of Melanophore Patterns in the Head Region of Chinook Salmon: A Natural Marker for the Reidentification of Individual Fish, North Am. J. Fish. Manag. 32 (2012) 806–816. doi:Doi 10.1080/02755947.2012.681014.

[24] M.F. Castanheira, M. Herrera, B. Costas, L.E.C. Conceição, C.I.M. Martins, Can We Predict Personality in Fish? Searching for Consistency over Time and across Contexts, PLoS One. (2013). doi:10.1371/journal.pone.0062037.

[25] M.F. Castanheira, M. Cerqueira, S. Millot, R.A. Gonçalves, C.C.V. Oliveira, L.E.C. Conceição, C.I.M. Martins, Are personality traits consistent in fish?-The influence of social context, Appl. Anim. Behav. Sci. (2016). doi:10.1016/j.applanim.2016.02.004.

[26] S. Millot, M.L. Bégout, B. Chatain, Risk-taking behaviour variation over time in sea bass Dicentrarchus labrax: Effects of day-night alternation, fish phenotypic characteristics and selection for growth, J. Fish Biol. (2009). doi:10.1111/j.1095-8649.2009.02425.x.

[27] S.S. Killen, S. Marras, M.R. Ryan, P. Domenici, D.J. Mckenzie, A relationship between metabolic rate and risk-taking behaviour is revealed during hypoxia in juvenile European sea bass, Funct. Ecol. (2012). doi:10.1111/j.1365-2435.2011.01920.x.

[28] D.C. Laursen, H. L. Olsén, M. de L. Ruiz-Gomez, S. Winberg, E. Höglund, Behavioural responses to hypoxia provide a non-invasive method for distinguishing between stress coping styles in fish, Appl. Anim. Behav. Sci. 132 (2011) 211–216.

doi:10.1016/j.applanim.2011.03.011.

[29] M.A. Vindas, M. Gorissen, E. Höglund, G. Flik, V. Tronci, B. Damsgård, P.-O. Thörnqvist, T.O. Nilsen, S. Winberg, Ø. Øverli, L.O.E. Ebbesson, How do individuals cope with stress? Behavioural, physiological and neuronal differences between proactive and reactive coping styles in fish, J. Exp. Biol. (2017). doi:10.1242/jeb.153213.

[30] C.E. Adams, F.A. Huntingford, J.F. Turnbull, C. Beattie, Alternative competitive strategies and the cost of food acquisition in juvenile Atlantic salmon (Salmo salar), Aquaculture. (1998). doi:10.1016/S0044-8486(98)00302-0.

[31] S. Rey, S. Boltana, R. Vargas, N. Roher, S. Mackenzie, Combining animal personalities with transcriptomics resolves individual variation within a wild-type zebrafish population and identifies underpinning molecular differences in brain function, Mol. Ecol. (2013). doi:10.1111/mec.12556.

[32] R.Y. Wong, M.S. Lamm, J. Godwin, Characterizing the neurotranscriptomic states in alternative stress coping styles, BMC Genomics. (2015). doi:10.1186/s12864-015-1626-x.

[33] M. Rosengren, P.O. Thörnqvist, S. Winberg, K. Sundell, The brain-gut axis of fish: Rainbow trout with low and high cortisol response show innate differences in intestinal integrity and brain gene expression, Gen. Comp. Endocrinol. (2018). doi:10.1016/j.ygcen.2017.09.020.

[34] A.J. Frost, A. Winrow-Giffen, P.J. Ashley, L.U. Sneddon, Plasticity in animal personality traits: Does prior experience alter the degree of boldness?, Proc. R. Soc. B Biol. Sci. (2007). doi:10.1098/rspb.2006.3751.

[35] T. Champneys, G. Castaldo, S. Consuegra, C. Garcia De Leaniz, Density-dependent changes in neophobia and stress-coping styles in the world's oldest farmed fish, R. Soc. Open Sci. (2018). doi:10.1098/rsos.181473.

[36] S.S. Killen, B. Adriaenssens, S. Marras, G. Claireaux, S.J. Cooke, Context dependency of trait repeatability and its relevance for management and conservation of fish populations, Conserv. Physiol. (2016). doi:10.1093/conphys/cow007.

[37] N. Grissom, S. Bhatnagar, Habituation to repeated stress: Get used to it, Neurobiol. Learn. Mem. 92 (2009) 215–224.

doi:10.1016/j.nlm.2008.07.001.

[38] J.S. Thomson, P.C. Watts, T.G. Pottinger, L.U. Sneddon, Plasticity of boldness in rainbow trout, Oncorhynchus mykiss: Do hunger and predation influence risk-taking behaviour?, Horm. Behav. (2012). doi:10.1016/j.yhbeh.2012.03.014.

[39] M. de L. Ruiz-Gomez, S. Kittilsen, E. Höglund, F.A. Huntingford, C. Sørensen, T.G. Pottinger, M. Bakken, S. Winberg, W.J. Korzan, Ø. Øverli, Behavioral plasticity in rainbow trout (Oncorhynchus mykiss) with divergent coping styles: When doves become hawks, Horm. Behav. (2008). doi:10.1016/j.yhbeh.2008.05.005.

[40] Y. Sakakura, K. Tsukamoto, Effects of density, starvation and size difference on aggressive behaviour in juvenile yellowtails (Seriola quinqueradiata), J. Appl. Ichthyol. (1998). doi:10.1111/j.1439-0426.1998.tb00607.x.

[41] G.E. Brown, J.A. Brown, R.K. Srivastava, The effect of stocking density on the behaviour of Arctic charr (Salvelinus alpinus L.), J. Fish Biol. (1992). doi:10.1111/j.1095-8649.1992.tb02722.x.

[42] D.C. Laursen, B.K. Larsen, P. V. Skov, E. Höglund, Improved growth performance in rainbow trout Oncorhynchus mykiss reared at high densities is linked to increased energy retention, Aquaculture. 442 (2015) 69–73. doi:10.1016/j.aquaculture.2015.02.035.

[43] C.E. Adams, J.E. Bron, J.F. Turnbull, A. Bell, F.A. Huntingford, Multiple determinants of welfare in farmed fish: stocking density, disturbance, and aggression in Atlantic salmon (Salmo salar), Can. J. Fish. Aquat. Sci. 64 (2007) 336–344. doi:10.1139/f07-018.

[44] S. Alfonso, B. Sadoul, M. Gesto, L. Joassard, B. Chatain, B. Geffroy, M.L. Bégout, Coping styles in European sea bass: The link between boldness, stress response and neurogenesis, Physiol. Behav. (2019). doi:10.1016/j.physbeh.2019.04.020.

[45] T.G. Pottinger, Context dependent differences in growth of two rainbow trout (Oncorhynchus mykiss) lines selected for divergent stress responsiveness, Aquaculture. 256 (2006) 140–147. doi:10.1016/j.aquaculture.2006.01.023.

[46] S.E. Fevolden, K.H. Røed, K.T. Fjalestad, J. Stien, Poststress levels of lysozyme and cortisol in adult rainbow trout: Heritabilities and genetic correlations, J. Fish Biol. (1999). doi:10.1006/jfbi.1999.0930.

[47] F.A.M. Volckaert, B. Hellemans, C. Batargias, B. Louro, C. Massault, J.K.J. Van Houdt, C. Haley, D.J. De Koning, A.V.M. Canario,

Heritability of cortisol response to confinement stress in European sea bass dicentrarchus labrax, Genet. Sel. Evol. (2012). doi:10.1186/1297-9686-44-15.

[48] M. Herrera, M.F. Castanheira, L.E.C. Conceição, C.I. Martins, Linking risk taking and the behavioral and metabolic responses to confinement stress in gilthead seabream Sparus aurata, Appl. Anim. Behav. Sci. (2014). doi:10.1016/j.applanim.2014.03.001.

[49] Ø. Øverli, W.J. Korzan, E. Höglund, S. Winberg, H. Bollig, M. Watt, G.L. Forster, B.A. Barton, E. Øverli, K.J. Renner, C.H. Summers, Stress coping style predicts aggression and social dominance in rainbow trout, Horm. Behav. (2004). doi:10.1016/j.yhbeh.2003.12.002.

[50] S. Wendelaar Bonga, The stress response in fish, Physiol. Rev. 77 (1997) 591–625. http://physrev.physiology.org/content/77/3/591.short.

[51] M. Gesto, M.A. López-Patiño, J. Hernández, J.L. Soengas, J.M. Míguez, The response of brain serotonergic and dopaminergic systems to an acute stressor in rainbow trout: a time course study., J. Exp. Biol. 216 (2013) 4435–42. doi:10.1242/jeb.091751.

[52] J.S. Thomson, P.C. Watts, T.G. Pottinger, L.U. Sneddon, HPI reactivity does not reflect changes in personality among trout introduced to bold or shy social groups, Behaviour. (2016). doi:10.1163/1568539x-00003398.

[53] J. Vaz-Serrano, M.L. Ruiz-Gomez, H.M. Gjøen, P. V. Skov, F.A. Huntingford, Øverli, E. Höglund, 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, Physiol. Behav. 103 (2011) 359–364. doi:10.1016/j.physbeh.2011.02.025.

[54] K. Boulton, E. Couto, A.J. Grimmer, R.L. Earley, A.V.M. Canario, A.J. Wilson, C.A. Walling, How integrated are behavioral and endocrine stress response traits? A repeated measures approach to testing the stress-coping style model, Ecol. Evol. (2015). doi:10.1002/ece3.1395.

[55] S. Winberg, E. Höglund, Ø. Øverli, Variation in the Neuroendocrine Stress Response, in: Fish Physiol., 2016. doi:10.1016/B978-0-12-802728-8.00002-3.

[56] M. Gesto, L. Madsen, N.R. Andersen, A. Jokumsen, Differences in stress and disease resilience related to emergence time for first feeding in farmed rainbow trout (Oncorhynchus mykiss), J. Exp. Biol. 221 (2018). doi:10.1242/jeb.174623.

[57] A.J. Emerson, D.P. Kappenman, P.J. Ronan, K.J. Renner, C.H. Summers, Stress induces rapid changes in serotonergic activity: Restraint and exertion, Behav. Brain Res. 111 (2000) 83–92. doi:10.1016/S0166-4328(00)00143-1.

[58] S. Winberg, A. Nilsson, P. Hylland, V. Söderstöm, G.E. Nilsson, Serotonin as a regulator of hypothalamic-pituitary-interrenal activity in teleost fish, Neurosci. Lett. 230 (1997) 113–116. doi:10.1016/S0304-3940(97)00488-6.

[59] S. Winberg, G.E. Nilsson, Roles of brain monoamine neurotransmitters in agonistic behaviour and stress reactions, with particular reference to fish, Comp. Biochem. Physiol. Part C Comp. 106 (1993) 597–614. doi:10.1016/0742-8413(93)90216-8.

[60] M. Bortolato, K. Chen, J.C. Shih, The Degradation of Serotonin: Role of MAO, in: Handb. Behav. Neurosci., 2010. doi:10.1016/S1569-7339(10)70079-5.

[61] C. Lillesaar, The serotonergic system in fish, J. Chem. Neuroanat. (2011). doi:10.1016/j.jchemneu.2011.05.009.

[62] Ø. Øverli, C. Sørensen, K.G.T. Pulman, T.G. Pottinger, W. Korzan, C.H. Summers, G.E. Nilsson, Evolutionary background for stress-coping styles: Relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates, Neurosci. Biobehav. Rev. 31 (2007) 396–412. doi:10.1016/j.neubiorev.2006.10.006.

[63] C. Magnhagen, T. Backström, Øverli, S. Winberg, J. Nilsson, M.A. Vindas, E. Brännäs, Behavioural responses in a net restraint test predict interrenal reactivity in Arctic charr Salvelinus alpinus, J. Fish Biol. (2015). doi:10.1111/jfb.12691.

Figure 1 Outline of the experimental procedures carried out from the arrival of the fish to the facilities until the end of the experimental work (see main text for more details). Black spots indicate mass measurements of L and W fish (see also Figure 4).

Figure 2 Average mass, before and after competition, and SGR of fish selected as L (losers) and W (winners) during the first competition trial. Columns and error bars indicate mean + SD of the mass and SGR values (first averaged per competition tank) of the fish selected as L or W (n=34 competitions). * indicates significant differences between L and W fish ($p < 0.001$).

Figure 3 Plasma concentration of cortisol, glucose and lactate in L (losers) and W (winners) fish just before (control) or 40 min after confinement (stress) (see the text for more details about the stress-challenge protocol). Data represent the mean + SD of n = 10-12 fish. * indicates a significant difference against the control, within competitor groups ($p < 0.001$).

Figure 4 Average mass of L and W fish after selection. Data represent mean + SD (W) or mean - SD (L) of n = 10 to 25 fish. The time scale indicates the days passed after the end of the fish selection process (see main text and Figure 1 for more details).

Figure 5 Performance of L and W fish during a second competition trial, carried out between 4 and 12 weeks after the first competition test. A: contribution of L and W fish to the losers (L') and winners (W') of the second competition. B: SGRs of L and W fish during the second competition. Data represent mean and SD (first averaged per competition tank) of n = 17 competitions.

Figure 6 Plasma concentration of cortisol, glucose and lactate in L' (losers) and W' (winners) fish just before (control) or 40 min after confinement (stress). The stress challenge trial was performed immediately after the second competition trial (see the text for more details). Data represent the mean + SD. Number of samples per group was variable, as follows: control-L'= 18; control-W' = 12; stress-L' = 19; stress-W' = 24. * indicates a significant difference against the control, within competitor groups ($p < 0.001$). # indicates a significant difference between L' and W', within stress group ($p < 0.001$).

Figure 7 Serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and the serotonergic mass ratio (5-HIAA/5-HT) in the telencephalon of L' (losers) and W' (winners) fish just before (control) or 40 min after confinement (stress). The stress challenge trial was performed immediately after the second competition trial (see the text for more details). Data represent the mean + SD. Number of samples per group was variable, as follows: control-L'= 18; control-W' = 12; stress-L' = 19; stress-W' = 24. * indicates a significant difference against the control, within competitor groups ($p < 0.001$).

Figure 8 Serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and the serotonergic mass ratio (5-HIAA/5-HT) in the brain stem of L' (losers) and W' (winners) fish just before (control) or 40 min after confinement (stress). The stress challenge trial was performed immediately after the second competition trial (see the text for more details). Data represent the mean + SD. Number of samples per group was variable, as follows: control-L'= 18; control-W' = 12; stress-L' = 19; stress-W' = 24. * indicates a significant difference against the control, within competitor groups ($p < 0.001$). # indicates a significant difference between L' and W', within stress group ($p < 0.01$).

Table 1. *F* and *p*-values of the 2-way ANOVAs assessing the effect of the factor "stress" (control: C, or stress: S), the factor "group" (winners: W or W', or losers: L or L') or their interaction, in experiments 1 and 2 (see the text for details). The effects were considered statistically significant at $p < 0.05$.

		Parameter	Stress		Group		Stress x Group	
			F and p values	*effect*	*F and p values*	*effect*	*F and p values*	*effect*
Exp. 1 Confinement challenge with L and W fish	plasma	cortisol	$F_{1,40} = 200.70$, $p < 0.001$	S > C	$F_{1, 40} = 0.115$, $p = 0.736$	-	$F_{1,40} = 0.057$, $p = 0.812$	-
		glucose	$F_{1,40} = 83.20$, $p < 0.001$	S > C	$F_{1,40} = 4.32$, $p = 0.044$	L > W	$F_{1,40} = 0.279$, $p = 0.600$	-
		lactate	$F_{1,40} = 301.08$, $p < 0.001$	S > C	$F_{1,40} = 0.176$, $p = 0.677$	-	$F_{1,40} = 0.579$, $p = 0.451$	-
Exp. 2 Confinement challenge immediately after a second competition test	plasma	cortisol	$F_{1,68} = 254.20$, $p < 0.001$	S > C	$F_{1,68} = 3.50$, $p = 0.066$	-	$F_{1,68} = 2.90$, $p = 0.093$	-
		glucose	$F_{1,68} = 38.89$, $p < 0.001$	S > C	$F_{1,68} = 1.80$, $p = 0.184$	-	$F_{1,68} = 1.41$, $p = 0.239$	-

		lactate	$F_{1,68}$ = 110.11, p < 0.001	see interaction	$F_{1,68}$ = 13.42, p < 0.001	see interaction	$F_{1,68}$ = 7.08, p = 0.011	within C, W' > L'
	telencephalon	5-HIAA	$F_{1,69}$ = 41.46, p < 0.001	S > C	$F_{1,69}$ = 0.011, p = 0.918	-	$F_{1,69}$ = 0.058, p = 0.811	-
		5-HT	$F_{1,68}$ = 0.018, p = 0.895	-	$F_{1,68}$ = 0.850, p = 0.360	-	$F_{1,68}$ = 0.005, p = 0.945	-
		5-HIAA/5-HT	$F_{1,68}$ = 59.74, p < 0.001	S > C	$F_{1,68}$ = 2.60, p = 0.111	-	$F_{1,68}$ = 0.042, p = 0.837	-
	brain stem	5-HIAA	$F_{1,69}$ = 44.36, p < 0.001	S > C	$F_{1,69}$ = 6.86, p = 0.011	L' > W'	$F_{1,69}$ = 1.08, p = 0.303	-
		5-HT	$F_{1,69}$ = 0.232, p = 0.632	-	$F_{1,69}$ = 0.053, p = 0.819	-	$F_{1,69}$ = 0.001, p = 0.972	-
		5-HIAA/5-HT	$F_{1,69}$ = 36.43, p < 0.001	S > C	$F_{1,69}$ = 6.21, p = 0.015	L' > W'	$F_{1,69}$ = 1.15, p = 0.287	-

Table 2. Behavioral parameters (mean ± SD) during confinement and net restraining tests, which were performed immediately after the second competition test. Data was analyzed based on the result of the second competition (L’ vs W’), but also based on the original selection group (L vs W). Comparisons were made by means of Student’s t-tests (or Mann-Whitney U tests, when normality or equality of variance assumptions were not fulfilled). * indicates a significant difference between competition groups (p < 0.01).

Confinement	**L'**	**W'**		**L**	**W**
opercular beat rate (min^{-1})	111.23 ± 8.62	112.00 ± 9.09		110.75 ± 11.31	112.83 ± 8.58
% of fish not moving	39.3	35.7		38.0	40.7
% time spent moving[a]	48.02 ± 35.92	47.55 ± 34.77		55.89 ± 36.05	48.68 ± 33.78
distance moved[a] (m)	12.15 ± 10.71	11.29 ± 9.05		14.95 ± 11.41	11.53 ± 8.47
average speed[a] ($m\ s^{-1}$)	0.11 ± 0.04	0.11 ± 0.05		0.11 ± 0.05	0.11 ± 0.05
Net restraining	**L'**	**W'**		**L**	**W**
weak movements (count)	17.71 ± 6.54	15.36 ± 11.29		16.87 ± 9.90	14.07 ± 5.74

strong movements (count)	30.43 ± 15.96	40.14 ± 14.58		29.19 ± 14.29	39.30 ± 14.21*****
total movements (count)	48.14 ± 15.13	55.50 ± 13.70		46.06 ± 17.71	53.37 ± 14.17
longer string of strong movements (count)	8.29 ± 3.56	9.79 ± 4.37		7.19 ± 2.98	10.61 ± 3.74*****
duration of initial pause (s)	13.34 ± 12.16	6.56 ± 5.45		12.77 ± 11.50	8.75 ± 7.34
number of pauses > 3s (count)	2.21 ± 1.25	2.50 ± 1.65		2.13 ± 1.38	2.53 ± 1.55
[a]: only in fish that moved					

Highlights

- Individual competitive ability was used to segregate rainbow trout coping styles
- Competitive ability was consistent over time but unrelated to acute stress responses
- The link competitive ability – growth performance depends on rearing conditions
- No performance or welfare advantages of segregation under aquaculture-like conditions

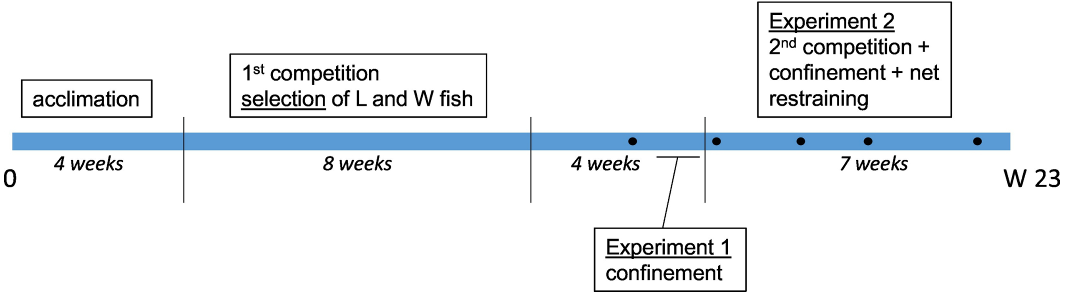

Experiment 2
2nd competition + confinement + net restraining
1st competition
selection of L and W fish
acclimation
4 weeks
8 weeks
4 weeks
7 weeks
0
W 23
Experiment 1
confinement

Figure 1

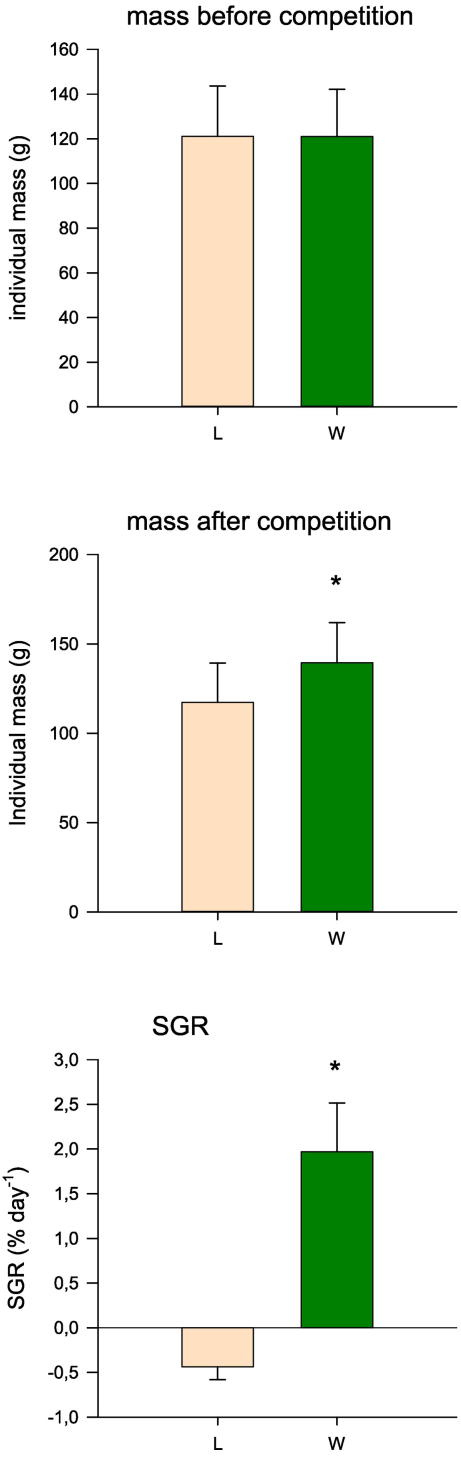

mass before competition
individual mass (g)
0
20
40
60
80
100
120
140
160
L
W
mass after competition
Individual mass (g)
0
50
100
150
200
*
L
W
SGR
SGR (% day^{-1})
-1,0
-0,5
0,0
0,5
1,0
1,5
2,0
2,5
3,0
*
L
W

Figure 2

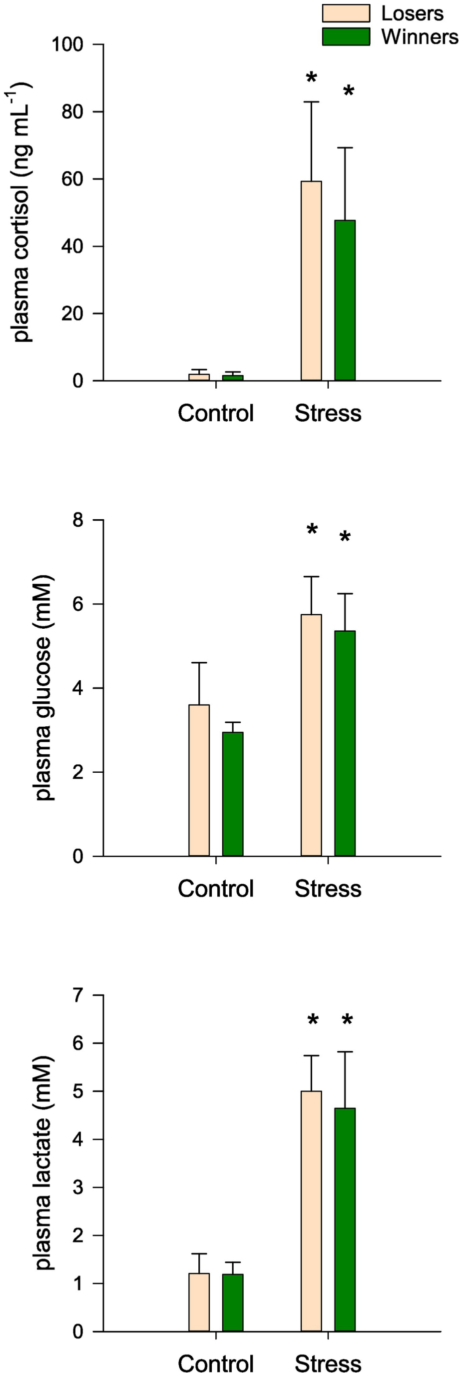
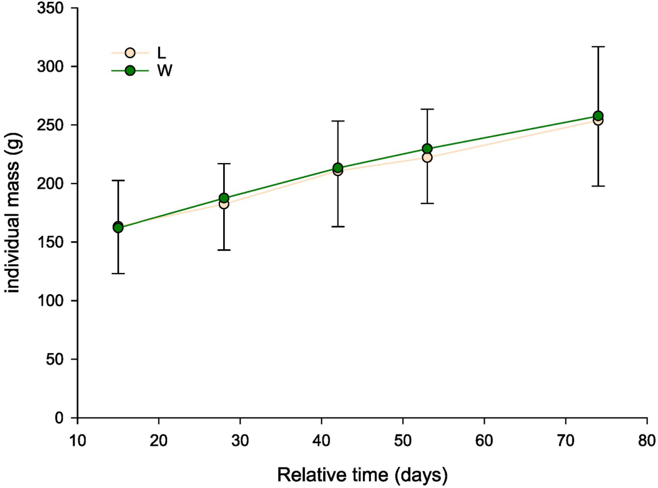

Losers
Winners
plasma cortisol (ng mL-1)
100
80
60
40
20
0
Control
Stress
plasma glucose (mM)
8
6
4
2
0
Control
Stress
plasma lactate (mM)
7
6
5
4
3
2
1
0
Control
Stress

Figure 3



350
300
250
200
150
100
50
0
individual mass (g)
L
W
10
20
30
40
50
60
70
80
Relative time (days)

Figure 4

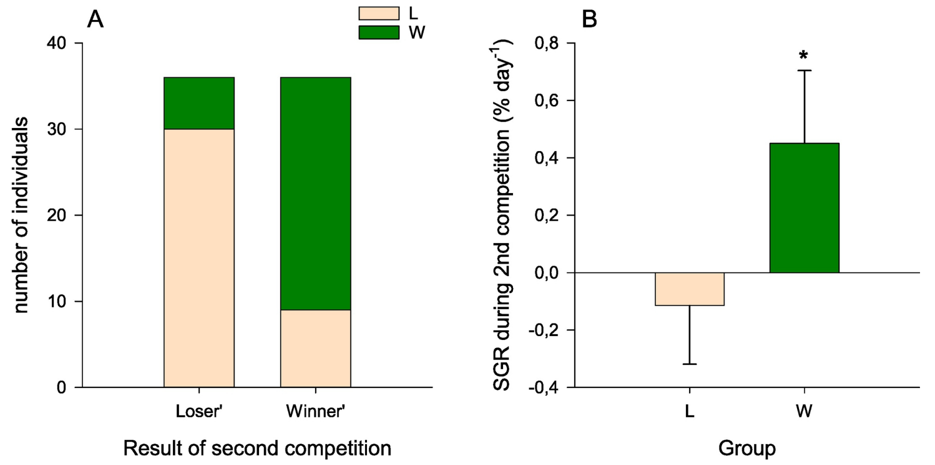

A
B
L
W
40
30
20
10
0
number of individuals
Loser'
Winner'
Result of second competition
0,8
0,6
0,4
0,2
0,0
-0,2
-0,4
SGR during 2nd competition (% day-1)
*
L
W
Group

Figure 5

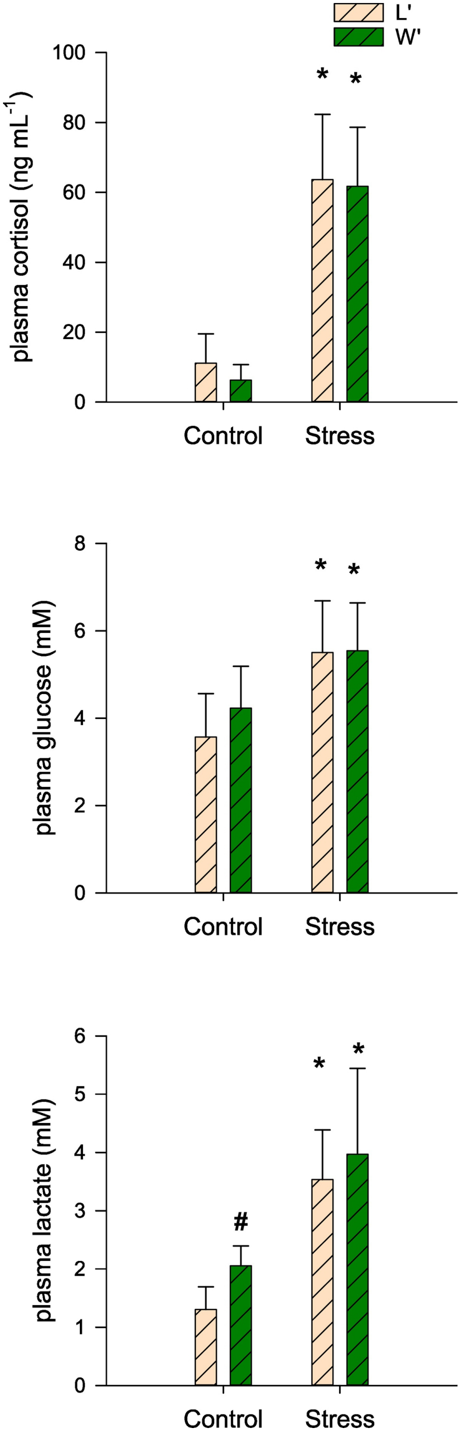

L'
W'
100
80
60
40
20
0
plasma cortisol (ng mL^{-1})
*
*
Control
Stress
8
6
4
2
0
plasma glucose (mM)
*
*
Control
Stress
6
5
4
3
2
1
0
plasma lactate (mM)
*
*
#
Control
Stress

Figure 6

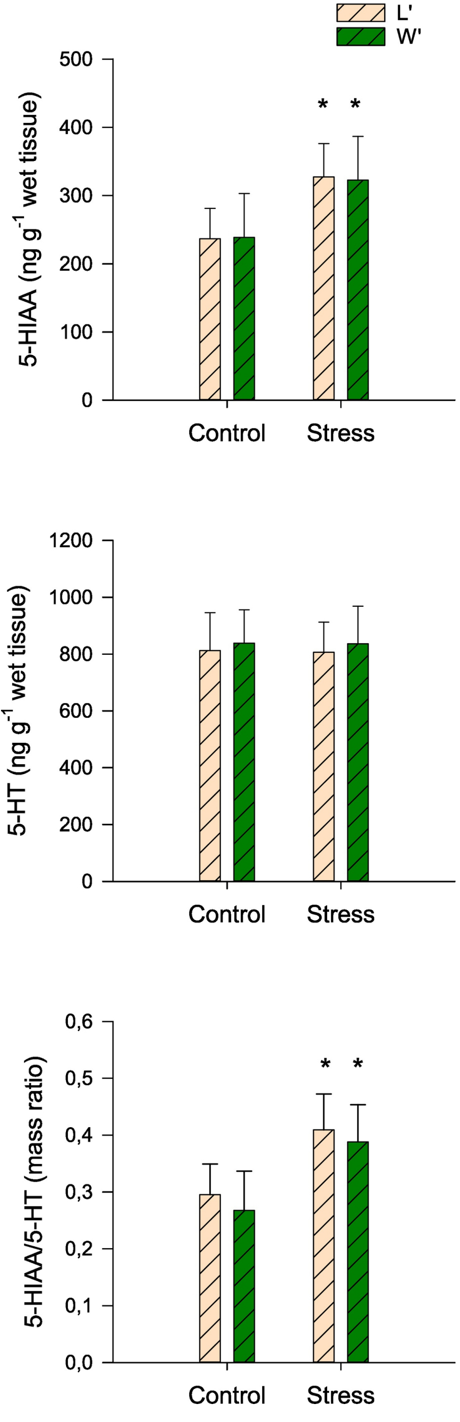
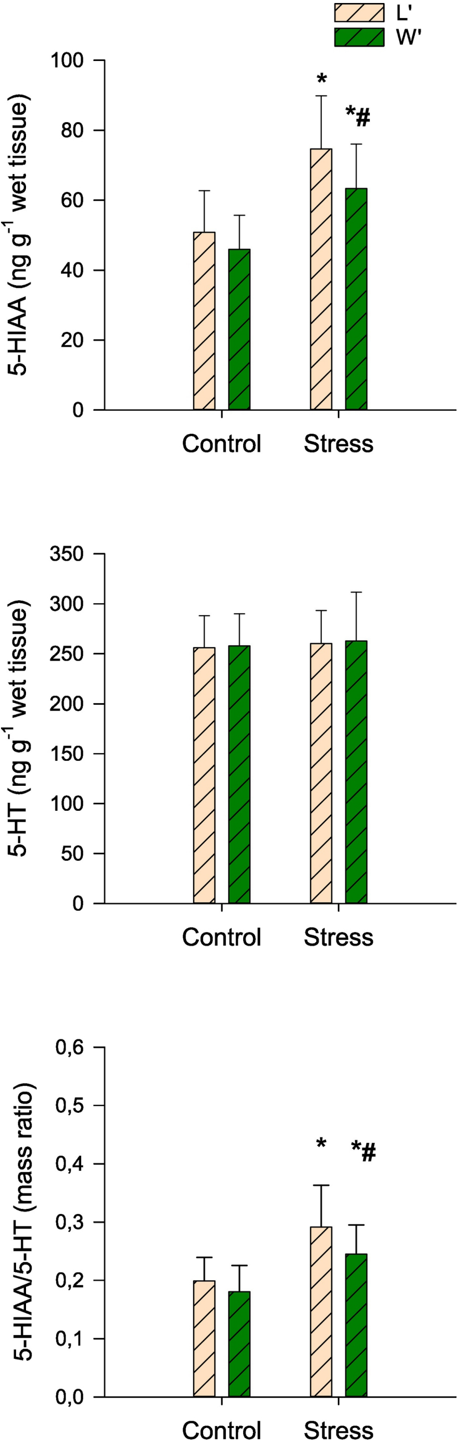

L'
W'
5-HIAA (ng g^{-1} wet tissue)
500
400
300
200
100
0
*
*
Control
Stress
5-HT (ng g^{-1} wet tissue)
1200
1000
800
600
400
200
0
Control
Stress
5-HIAA/5-HT (mass ratio)
0,6
0,5
0,4
0,3
0,2
0,1
0,0
*
*
Control
Stress

Figure 7



L'
W'
100
80
60
40
20
0
5-HIAA (ng g-1 wet tissue)
*
*#
Control
Stress
350
300
250
200
150
100
50
0
5-HT (ng g-1 wet tissue)
Control
Stress
0,6
0,5
0,4
0,3
0,2
0,1
0,0
5-HIAA/5-HT (mass ratio)
*
*#
Control
Stress

Figure 8