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Context-dependent responses to novelty in Rainbow trout (*Oncorhynchus mykiss*), selected for high and low post-stress cortisol responsiveness

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ABSTRACT

Previous studies in a rainbow trout model, selectively bred for high (HR) and low (LR) post stress plasma cortisol levels, have yielded data that are indicative of contrasting stress coping styles. Fish from the HR line have been suggested to display a more diverse behavioral repertoire in challenging situations than the LR counterpart. The present study addressed whether such variation in behavioral flexibility traits was evident in different experimental settings using these selection lines. The fish were subjected to three sets of challenges (novel object test, resident–intruder test and confinement stressor test), all which were repeated a week later. Introducing a novel object evoked a divergent behavioral response in association with feeding: fish from the LR line displayed consistently suppressed feed intake while the HR fish remained unaffected. This observation was found to be repeatable along with attack latency and movement activity from the resident–intruder and confinement stressor tests. These results indicate that the behavioral responses in this animal model are context-dependent and shed new light on the expression of behavioral flexibility.

1. Introduction

It is well known that individuals within a population often respond differently to challenges [1–4]. This intraspecific variation often comprises suites of behavioral and physiological traits that appear to be consistent over time as well as across situations. Behavioral ecologists and ethologists often refer to “behavioral syndromes”, “temperament” or “personality” to characterize this phenomenon, whereas researchers in stress physiology have promoted the term “coping styles” to describe such consistency in physiological and behavioral traits [1,2,5–10].

The behavioral responses to a challenge vary along a proactive–reactive continuum. Proactive individuals are characterized by high levels of aggression, active avoidance behavior and they more readily take risks. In contrast, reactive individuals show low levels of aggression, respond with immobility and are also less susceptible to take risks, but their behavioral repertoire are more flexible, to aversive stimuli (reviewed by Koolhaas et al. [10]). Both empirical studies [11,15–18] and the theoretical framework [6,9] suggest that behavioral flexibility is an important underlying component of coping styles. In a recent review, Coppens et al. [9] expanded the concept of stress coping, and suggested that the extent to which behavior is governed by environmental stimuli is an integral component of stress coping styles. A low behavioral flexibility can thus be attributed to individuals who pay little attention to changes in the environment and are prone to routine formation in coping with challenges. Behaviorally flexible individuals on the other hand seem to be highly attentive and able to readjust their behavior in the same circumstances.

The field of research regarding genetic/non-genetic factors involved in shaping personality traits is quite complex. For this reason, many investigators are using animal models, generated through selective breeding for specific phenotypes, in order to gain more insight on the causal mechanisms underlying individual variation. Over the last 15 years, several studies have emerged which are consistent with the hypothesis that stress coping styles, as it is described for mammals [5], also are present in fish [20–25,33]. The establishment of selection lines in rainbow trout (*Oncorhynchus mykiss*), with consistently high (HR) or low (LR) post-stress cortisol levels [26] has provided a unique model for further exploring the heritable variation in physiological and behavioral traits in teleosts. For example, behavioral studies from these trout lines have revealed that LR fish not only become socially dominant over HR in dyadic encounters, but also resume feeding earlier and display greater boldness after transfer to a novel environment [20,24,27]. Although it has been suggested that stress reactivity and concomitant differences in glucocorticoid release may not always co-vary with the coping style-axis [10], the HR/LR trout model shows remarkable similarities with the characteristics of proactive/reactive coping. In addition to the reported differences in social dominance and boldness, a recent study performed by Ruiz-Gomez et al. [18] indicates that the trout lines also differ in the propensity to follow and develop routines. The

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researchers observed that the LR fish obtain food at a slower rate compared to fish from the HR line when the feed source is relocated within an open field environment. However, this pattern was reversed when a novel object was presented adjacent to the relocated feed source; the HR individuals were now the ones who spent the longest time obtaining the food. The authors concluded that the HR line exhibited a reactive coping style with higher degree of behavioral flexibility whereas the LR counterpart appeared proactive and seemed to rely more on routines when they responded to subtle environmental changes [18]. In a comparable study, Sluyter et al. [19] observed that the relationship between coping style and behavioral flexibility was context dependent for mice in a shock-probe/defensive burying test. In short, they found that genetically selected nonaggressive and aggressive mice differed in their behavioral response to the shock-probe in an unfamiliar environment. However, the behavioral difference was no longer apparent when the test was performed in a familiar environment.

Since these context dependent responses in coping style were described in behaviorally selected mice [19], we wanted to determine if such phenomenon is also present in animals with divergent stress reactivity. To achieve this we extended the work by Ruiz-Gomez et al. [18] and investigated if the same stimuli, that induced inhibition in feeding behavior among reactive HR fish, evoked a similar behavioral response pattern when it was introduced in a more familiar home environment using the same trout model. Given that LR fish have previously been documented to become dominant over HR in size-matched encounters [24], we also wanted to find out if these lines differed in their aggressiveness toward a smaller, unselected intruder fish. In order to evaluate if potential differences in behavior are consistent over time, we repeated these tests on the same individuals one week later. An acute confinement stressor challenge was also performed to conclude the tests in order to ensure that both selection lines still displayed the divergence in post stress cortisol responsiveness.

2. Materials and methods

2.1. Experimental animals

The experiment was performed at the National Institute of Aquatic Resources in Hirtshals, Denmark (The Technical University of Denmark) during the autumn of 2008. The animals used in the present study were juvenile rainbow trout (*O. mykiss*) from the F4-generation (HR: N = 8, mean weight 100.8 ± S.E.M. 8.9 g and LR: N = 10, mean weight 107.9 ± S.E.M. 3.0 g). The breeding program for generating the HR and LR lines has previously been described by Pottinger and Carrick [24,26]. Prior to the experiments, the HR and LR fish were kept indoors in two separate 1000-liter holding tanks, which were continuously supplied with unchlorinated tap water at ambient temperature (varying between 10 and 14 °C). The fish were fed with commercial dry feed pellets (BioMar) once a day at 1.5% of the biomass in the tank.

2.2. Experimental conditions

The experiments were carried out in plastic observation aquaria (40 cm in length, 30 cm in width and height, a volume of 36 l). Each aquarium was covered with black plastic sheets on 3 sides to minimize disturbance from the surroundings. Light was provided by fluorescent tubes placed 1 m above the aquaria and the light/dark regime was set at 12/12 h with lights on at 8:00 to 20:00. The aquaria were continuously supplied with unchlorinated tap water (0.5 l/min) at ambient temperature (10–14 °C). This tank system was used for acclimation of the fish, novel object test and for the resident–intruder test, whereas separate transparent confinement boxes, with a volume of 5 l, were used for the acute stressor test. The sides of the boxes were covered with gray plastic walls except the front which allowed us to film the behavioral response.

2.3. Experimental protocol

Experimental fish were netted randomly and transferred from holding tanks to the observation aquaria, where they were allowed to acclimate to the new environment and feeding routines in social isolation. Fish were hand fed and offered feed 3 times a day; in the morning 08:00–10:00, midday 13:00–15:00 and evening 17:00–19:00 h. The fish were considered to be acclimated when they ate during all meals for 3 days in a row, referred to as acclimation time. There were no significant differences in the number of meals required before the fish began feeding between the selection lines; mean ± standard error of mean (S.E.M.), Mann Whitney *U*-test: HR = 2.6 ± 0.8 and LR = 3.1 ± 1.0, Z = −0.04, p = 0.96.

Following acclimation, the experiment comprised two phases: quantification of baseline feeding for six days followed by three days of challenge testing (Fig. 1). These two phases were repeated once, resulting in an experimental period of 18 days. Baseline feeding was calculated as the average morning-, midday- and evening feed intake. The fish were subjected to three different challenges during a three-day period beginning with a novel object test, followed by a resident–intruder test and finalized by a confinement test. All behavioral observations were carried out from video recordings. During the experiment, fish were offered 4 mm dice formed cubes of wet feed that comprised of a mixture containing herring and prawns, see [28] for more info. Since the water content was approximately 15 times higher in this type of diet compared to commercial feed the fish were fed up to 5% of their bodyweight per meal, thus in total 15% on a daily basis [28].The gross energy density in dry matter was similar in the commercial trout feed and herring/prawn mixture [28]. Apart from a few meals all fish fed until satiety rather than up to the preset maximum limit. Feed intake was quantified for each individual during every meal by weighing the amount of feed in the container prior to and after feeding. Feed was offered one piece at the time until the fish had rejected 3 pieces in a row. Unconsumed feed was immediately removed from the aquaria with a siphon.

2.4. Novel object test

On the morning prior to feeding the fish was exposed to a novel object [18] by dropping a yellow rubber stopper, 5 cm^3 in size, in the center of their home aquaria. The object was left in the aquaria for 10 min during which behavior was monitored. Behavioral parameters such as the total number of times approaching within 5 cm of the novel object as well as the time spent within this 5 cm-zone were quantified for each individual fish. In addition, locomotor activity, measured as time spent moving, was also measured during this time frame. This last parameter was defined as moving the body at least one head length (from operculum to nose) in any direction combined with active flapping of the tailfin. The feeding response in presence of the novel object was also assessed following 10 min of exposure and expressed as percentage of baseline feeding (i.e. the ratio between individual and baseline feed intake in both the HR and LR groups respectively). Prior to the tests, there were no significant differences between the lines in daily feed intake during quantification of the two rounds of baseline feeding (*t*-test; round one $t_{(16,\ 2)} = 1.07$, p = 0.29; round two $t_{(16,\ 2)} = -0.35$, p = 0.73). Mean daily feed intake during the first round of baseline feeding was 5.4 ± 0.7 g in HR and 4.4 ± 0.5 g in LR fish, and during the second round it was 6.9 ± 3.2 g in HR fish, 7.3 ± 2.6 g in LR fish (mean ± S.E.M). The feeding response to the stimuli was measured in the morning, 10 min after the novel object was dropped into the aquaria. The object was removed once the fish had rejected 3 pieces of offered feed in a row during this meal. In addition, feed intake was monitored at midday and evening meal to assess potential long term changes in the resumption of feeding after interaction with the novel object. The same object was used when the test was repeated 9 days later during the morning.

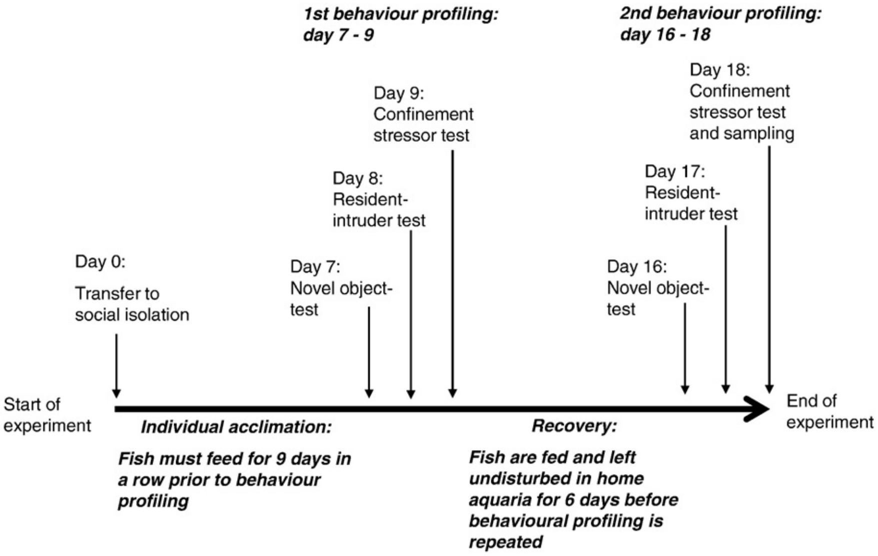

Fig. 1. Overview of the experimental design, see text for details.

2.5. Resident–intruder test

On the next day, the fish was exposed to a resident–intruder test [29]. A smaller conspecific, ranging from 30 to 50% in body mass of the resident fish, was introduced into the home aquaria during the morning. Both fish were allowed to interact with each other for 10 min following the first attack. The behavior of the resident fish was videotaped for later analysis of aggression. From the video recordings, the latency to first attack by the resident fish was measured. Attacks were defined as either chases and/or bites and each intruder was used only once. Fish that did not display any signs of aggression were assigned an attack latency of 600 s and number of attacks equal to 0.

2.6. Confinement test

On the final day of challenge testing the fish were exposed individually to a standardized confinement stressor during the morning by transferring them to confinement boxes. The fish were confined for half an hour and their behavior (locomotor activity, measured as time spent moving,) was recorded during the first 10 min following transfer. This timeframe was chosen as the HR and LR lines have recently been reported to differ in locomotor activity during this period when exposed to confinement stress [30]. After completion of the confinement the fish were put back into their home aquaria for recovery in 6 days before the tests were repeated again. Locomotor activity was defined as described in the novel object test and quantified from the video recordings.

Immediately after the second round of confinement the fish were killed within 30 s by a lethal dose of anesthesia (0.5 g/l tricaine methanesulfonate, MS 222). Approximately 1 ml blood was withdrawn from the caudal vein with a syringe, pre-treated with EDTA. Plasma samples were separated after centrifugation for 5 min at 5000 rpm, frozen on dry ice and stored at −80 °C for later cortisol analysis.

2.7. Plasma cortisol assay

Plasma cortisol levels were analyzed using radioimmunoassay, modified from Pottinger and Carrick [24]. Briefly, the samples were extracted in ethyl acetate, evaporated and redissolved in phosphate buffered saline. Aliquots of anti-cortisol antibody were used in the proportion of 1:200 in all tubes (except totals and blanks). Concentrations of plasma cortisol in unknown samples were calculated using a polynomial function generated from the standard curve. A subset of samples were also spiked with known amounts of $[H^3]$-cortisol in quadruplicate in order to check for losses due to extraction. The resulting average recovery was 62%, which was accounted for to all samples. All samples were run in a single assay and the minimal detection limit was 0.1 ng ml^{-1}, while the intra-assay variation was 0.7%.

2.8. Data analysis

Mean daily feed intake during baseline feeding was analyzed using t tests. All other observations were analyzed separately for each test round using the non-parametric Mann–Whitney *U* test, since neither the assumptions of normality nor homogeneity in variance (Shapiro–Wilk and Levene's test) were met in any of the data-sets. A Bonferroni procedure was applied for the novel object test to assess potential changes in appetite over the 3 meal sessions among the selection lines. Correlations in behavior between both test rounds were also investigated for repeatability within individuals using the Spearman rank correlation test. All data (presented as median or mean ± S.E.M.) were analyzed using Statistica (StatSoft Inc., Tulsa, OK, U.S.A.) and the significance level was set at $\alpha = 0.05$ unless stated otherwise (see the Results section for more info).

3. Results

3.1. Behavioral responsiveness during novel object test

Locomotor activity, number of times approaching within 5 cm of the novel object and the time spent within this zone is presented in Table 1. No behavioral differences were detected in any of the measured variables: round one; locomotor activity (LR = 41 s, HR = 74 s, $Z = 0.71$, $p < 0.48$), number of times entering the novel object zone (LR = 11, HR = 13, $Z = 0.85$, $p < 0.40$), time spent within the novel object zone (LR = 110 s, HR = 126 s, $Z = -0.09$, $p < 0.95$) and round two; locomotor activity (LR = 20 s, HR = 36 s, $Z = 0.44$, $p < 0.66$), number of times entering the novel object zone (LR = 8, HR = 11, $Z = 0.84$, $p < 0.40$), time spent within the novel object zone (LR = 332 s, HR = 70 s, $Z = 1.60$, $p < 0.11$). Neither were there any significant correlations in behavior between the rounds: locomotor activity ($r_s = 0.34$, $p < 0.17$), number of times entering the novel

Table 1
Results from the behavioral tests in LR (N = 10) and HR (N = 8) trout. All variables are presented as medians. Asterisks* indicates statistically significant correlation between 1st and 2nd test round (Spearman rank test, $p<0.05$).

Stressor test	Selection line	1st test	Mann–Whitney *U* test	2nd test	Mann–Whitney *U* test	Spearman rank correlation test (1st vs. 2nd test)
Novel object						
Locomotor activity (s)	LR	41	$Z=0.71$, $p<0.48$	20	$Z=0.44$, $p<0.66$	$r_s=0.34$, $p<0.17$
	HR	74		36		
No of times approaching within 5 cm of the object	LR	11	$Z=0.85$, $p<0.40$	8	$Z=0.84$, $p<0.40$	$r_s=0.09$, $p<0.72$
	HR	13		11		
Time spent within 5 cm of the object (s)	LR	110	$Z=-0.09$, $p<0.93$	332	$Z=-1.60$, $p<0.11$	$r_s=0.09$, $p<0.71$
	HR	126		70		
Resident intruder						
Latency to 1st attack (s)	LR	315	$Z=-1.08$, $p<0.28$	285	$Z=-0.41$, $p<0.68$	$r_s=0.61$, $p<0.01$*
	HR	205		183		
Total number of attacks	LR	14	$Z=0.99$, $p<0.32$	21	$Z=-0.77$, $p<0.44$	$r_s=0.27$, $p<0.29$
	HR	21		14		
Confinement						
Locomotor activity at 0–10 min (s)	LR	63	$Z=-0.98$, $p<0.33$	46	$Z=0.18$, $p<0.86$	$r_s=0.58$, $p<0.01$*
	HR	37		53		

object zone ($r_s=0.09$, $p<0.72$) and the time spent within this zone ($r_s=0.09$, $p<0.71$)

In the first round of the novel object test (Fig. 2A), the LR line displayed a decrease to 33 ± 17% of baseline feed intake whereas the HR appeared unaffected (91 ± 12%), when a novel object was presented into their home aquaria during the morning ($Z=2.49$, $p<0.012$). Following removal of the novel object, strain differences in feed intake were no longer apparent during midday or evening ($Z=-0.71$, $p<0.48$ and $Z=-0.80$, $p<0.42$ respectively). In the second novel object test (Fig. 2B) both lines reacted similarly as in the first round, with the LR fish decreasing their feed intake to 13 ± 11% as opposed to 83 ± 22% seen among the HR ($Z=2.44$, $p<0.015$) during the morning. No differences in feed intake between the LR- and HR-fish were found during midday- ($Z=-1.11$, $p<0.27$) or evening-feeding ($Z=1.10$, $p<0.29$).

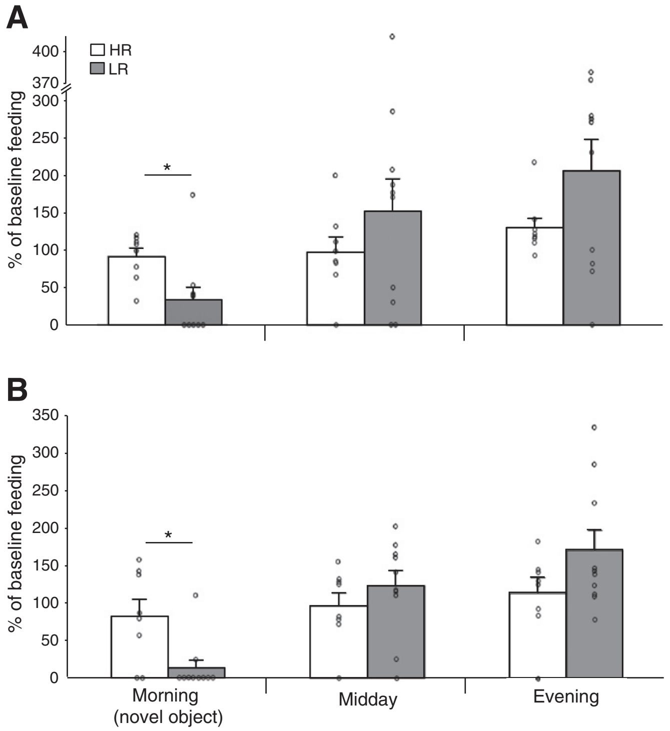

Fig. 2. Percentage of baseline feeding (mean + S.E.M.) in HR- and LR trout in presence of (morning) or without (midday and evening) a novel object in the observation aquaria. The test was performed on day 7 (A) and repeated on day 16 (B), see text for more details. Asterisks* indicates statistically significant difference between selection lines (Mann–Whitney *U* test with Bonferroni correction, $p<0.017$).

According to Bonferroni correction, which required a p-value of $\alpha<0.017$ for statistical significance, our obtained p-values during the morning in both test rounds fell below this limit. The Spearman rank test revealed a significantly positive correlation between the two rounds regarding changes in feed intake upon introduction of the novel object ($r_s=0.49$, $p<0.037$).

3.2. Resident–intruder test and confinement challenge

Table 1 summarizes the results on the behavioral measurements from the resident–intruder test and acute confinement challenge. No line differences were observed in the latency to first attack in either test round (1st round; LR = 315 s, HR = 205 s, $Z=-1.08$, $p<0.28$ and 2nd round; LR = 285 s, HR = 183 s, $Z=-0.41$, $p<0.68$) or total number of aggressive acts towards an intruder (1st round; LR = 14 attacks, HR = 21 attacks, $Z=0.99$, $p<0.32$ and 2nd round; LR = 21, HR = 14, $Z=-0.77$, $p<0.44$). Nor were any differences detected in the locomotor activity during both acute confinement stressor tests (1st round; LR = 63 s, HR = 37 s, $Z=-0.98$, $p<0.33$ and 2nd round; LR = 46 s, HR = 53 s, $Z=0.18$, $p<0.86$). However, a significant positive correlation was found between both test rounds in the latency to first attack ($r_s=0.61$, $p<0.007$) and locomotor activity ($r_s=0.58$, $p<0.01$). No such relationship was detected in the total number of aggressive acts ($r_s=0.27$, $p<0.29$).

The confinement challenge test did evoke a difference in the endocrine stress response during which HR displayed higher plasma cortisol levels than the LR fish (Fig. 3. $Z=2.31$, $p<0.021$), confirming that both lines maintain divergent post-stress cortisol levels.

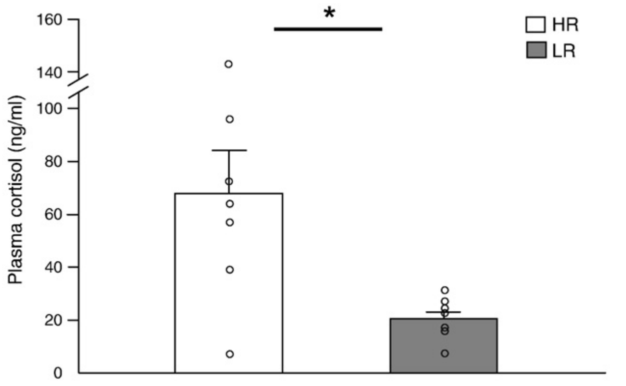

Fig. 3. Plasma cortisol levels (mean + S.E.M.) in HR- and LR trout after 30 min of 2nd confinement. Asterisks* indicates statistically significant difference (Mann–Whitney *U* test, $p<0.05$).

4. Discussion

In the study presented herein, we demonstrated consistency over time in feeding responses in the presence of a novel object, aggression towards a smaller intruder as well in the behavioral responses to confinement in selected rainbow trout lines. Among these traits, introducing a novel object induced suppressed feeding behavior in the LR line, whereas HR fish appeared to be unaffected by this stimuli.

These results seem to be in direct contrast to the findings by Ruiz-Gomez et al. [18], who reported that behavioral inhibition was most pronounced among HR fish rather than LR when feed was made accessible in the presence of a novel object. However, in that study protective shelters were available during the tests and the object was presented in an open area which the fish had to pass in order to enter the feeding station. In our model, the novel object constituted an inescapable challenge as it was introduced in an environment where the fish had been fully acclimated without a refuge. This suggests that the contrasting behavioral responses towards the novel object between our study and the one performed by Ruiz-Gomez et al. [18] are related to differences in context, in which the stimulus was presented.

In the study by Ruiz-Gomez et al.[18] LR fish did not only seem unaffected by this mild stressor, but they also swam over the visible feed source which was relocated adjacent to the novel object and went directly for a previously learned reward station. HR fish, on the other hand, adjusted their behavior and were slower to feed when confronted with the same object. Similar differences in behavioral flexibility have been documented among rodent models. Using mice strains selected for short (SAL) and long attack latency (LAL) to an intruder, Benus et al. [11] found that SAL mice took longer time and more trials to readjust their path to a feeding station in a reversal learning task. Furthermore, studies in mice originating from unselected populations has shown that SAL males are less sensitive to environmental changes (i.e. introducing a novel object) in reaching their feed reward [14]. In light of the results by Ruiz-Gomez et al. [18], who observed that HR fish took longer time to find feed when confronted with a novel object, we demonstrated such disruption in feeding behavior in the LR line in response to the same stimuli. It is possible that the LR trout were distracted in their feeding routine by the novel object in our study, and that the sensitivity to environmental changes is context dependent in these two lines.

Generally, the effects of novelty on the foraging behavior of HR and LR trout are elusive. Based on gut content after seven days of isolation, fish from the LR line have been suggested to acclimate more rapidly than HR fish under similar conditions as in our experiment [27]. On the other hand, behavioral studies report that the HR fish can resume feeding faster after isolation than the LR fish [18,39]. However, in our study we found no differences in acclimation time between the lines following transfer from holding tanks to the observation aquaria. Previous studies in rainbow trout demonstrate that behavioral reactions to novelty can be affected by previous experience. In the HR–LR trout model, severe stressors such as transportation, crowding, starvation and novel environment has been shown to evoke a reversal in behavioral profile in terms of feeding resumption and aggression [15]. Moreover, Frost et al. [32] found that behavioral responses to a novel object were influenced by previous social experience. It is possible that the inconsistencies in data on feeding responses to novelty in studies using the HR-LR trout model may be related to differences in previous experience. In order to minimize such effects we considered the fish fully acclimated to their new environment after fulfilling the criteria of feeding three days in a row, instead of allowing the animals a fixed habituation period for one week, which is standard in similar experiments investigating behavior of isolated fish [28,36–38].

In our study, we did not observe any differences in behavioral responses in presence of the novel object prior to offering feed. These results are very much in agreement with previous work on these lines, reporting no clear differences in behavior between isolated HR and LR trout when subjected to a novel object test [20,35]. However, this is somewhat in contrast to similar studies using another animal model, mice lines selected for high (HR) or low (LR) post stress corticosterone, which show differences in exploratory behavior in novel situations [12]. In our study divergent behavioral responses to novelty became apparent only in association with feeding in fish fully acclimated to their environment, supporting the suggestion that these differences are more related to routine formation and behavioral flexibility than a reaction to novelty alone. However, it cannot be excluded that the suppression of feeding in LR trout was just a response to the presence of the novel object. One might argue that results from this study as well as those by Ruiz-Gomez et al. [18] could be interpreted as the LR trout being more likely to suppress feeding in response to a challenge.

Locomotor activity during the first 10 min of confinement stress showed consistency over time but did not differ between the two lines in our study. This is in contrast to previous work where the HR and LR trout lines have been reported to differ in the time spent moving during the first 10 min of confinement stress [30]. Furthermore, no differences in agonistic behavior were found between HR and LR trout either in the latency to first attack or in number of aggressive acts performed in the resident–intruder tests. Earlier studies have shown that LR fish becomes socially dominant over HR fish [24], suggesting that LR fish are more aggressive than HR fish. However, our data indicate that the two lines do not differ in the level of aggression. Similar results have previously been reported in a study by Schjolden et al. [20], in which exposure to a smaller conspecific did not indicate any clear differences in aggression between the two lines. This could be due to the smaller size of the intruder, which is unlikely to engage in agonistic confrontations but rather adopt a submissive role very quickly. The aggressive behavior among the resident fish is also influenced by the varying behavior of the intruder fish, which ranges from total immobility to erratic panic swimming. Using smaller conspecifics rather than size-matched pairings in these tests is intentional since such a design is less biased by the different suites of behaviors displayed by the intruders themselves. In our study, attack latency showed a positive correlation between the first and second round of intruder testing, suggesting that this measure was related to aggression of the resident fish and that aggression was a trait showing consistency over time.

In rodent models, SAL- and LAL mice lines demonstrate a strong relationship between hypothalamic–pituitary–adrenal (HPA) axis reactivity and aggressive behavior [34]. The rainbow trout lines used in our study were selected for cortisol responsiveness to a standardized stressor and it is possible that such a selection regime results in a slightly different association between traits forming contrasting stress coping styles. The HR/LR mice model share some similarities with the HR/LR trout model, such as the propensity to aggression in LR mice and social dominance in LR trout, but they differ in other parameters. For instance LR trout has been observed to display higher exploratory activity than HR fish in novel open environments [20], whereas an opposite behavioral pattern is evident in the corresponding rodent model, during similar contexts such as open field- and elevated platform tests [12]. In other words, individuals selected for low stress reactivity (LR) in the trout and mice models exhibit behaviors indicating different stress coping styles when exposed to aversive stimuli. Mice from the LR line appear to display traits towards the reactive coping gradient [12,13], whereas LR trout seem to express a more proactive behavior. Since these two animal models have been generated on the same physiological basis and yet display discrepancies in behavioral phenotypes, this illustrates the rather complex relationship between HPA/HPI axis reactivity and the proactive–reactive continuum (reviewed by Koolhaas et al. [10]).

Although the LR and HR trout lines are selected on stress reactivity they display divergent phenotypes resembling proactive and reactive stress coping styles, respectively [18,20,24,27]. Furthermore, the results by Ruiz-Gomez et al. [18] are in line with the concept of behavioral flexibility as one of the underlying factors discriminating the two stress coping styles [9,10]. According to that framework, reactive individuals are more able to readjust their behavior when confronted with an environmental challenge than the proactive counterpart [5,31]. However, the results from our study demonstrate that a novel object, presented in a different context than in the study performed by Ruiz-Gomez et al. [18], can interrupt feeding routines in the LR line, which is generally believed to express more of a proactive profile [20,24,25,27]. This could be interpreted as the LR fish adopting a more flexible behavior, which in turn implies that behavioral flexibility is context dependent. Contexts such as familiarity have previously been shown to induce behavioral trait-changes in the proactive–reactive continuum among rodents. Using the SAL/LAL mice model, Sluyter et al. [19] found that individuals from the latter line could respond either with immobility or active defensive burying when they were shocked by an electrified probe. These two distinct behavioral patterns were evident when the stressor was applied in an environment with alien sawdust but absent in the presence of familiar cues (home cage sawdust), suggesting that LAL mice are behaviorally more flexible than the SAL line. In light of those results, it is possible that differences in familiarity caused the opposite pattern in feeding behavior during the novel object test between our study and the study performed by and Ruiz-Gomez et al. [18]. Earlier work on F3- and F4 generations in the HR–LR trout model has demonstrated that behavioral traits which may be indicative of proactive and reactive coping styles, in terms of feeding resumption and social dominance, can be reversed without altering the divergence in post stress corticosteroid responsiveness [15]. This provides further support to that some of the individual variation in behavior is context dependent, and which do not always co-vary with stress responsiveness.

In conclusion, this study demonstrates that attack latency, locomotor activity during exposure to confinement and feeding response to novelty stressors are repeatable traits in the HR and LR trout lines. Among these behavioral characteristics, which have been associated with the proactive/reactive stress coping styles, the suppressed feeding rate in presence of the novel object was the only trait separating the HR and LR trout lines. The finding that LR trout adjusted their behavior to a novel stimulus, by decreasing the feed intake, indicates that the behavioral responses in this trout model are context-specific and may even suggest a more flexible behavior in this selection line, than previously reported. Since these rainbow trout lines, which were selected on the basis of their post stress cortisol responsiveness, displayed a somewhat contrasting behavioral pattern compared to a recent study [18] further supports the idea that coping styles and stress reactivity can be uncoupled.

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