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Divergence in behavioural responses to stress in two strains of rainbow trout (*Oncorhynchus mykiss*) with contrasting stress responsiveness

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Abstract

The aim of this study was to establish whether two lines of rainbow trout divergent for their plasma cortisol response to a standardized stressor would show consistent differences in their behavioural response to a range of challenging situations. Our results show that the high- and low-responding (HR and LR) lines of rainbow trout did not differ in the aggression shown towards an intruder or in their response to the introduction of a novel object to their home environment. However, there was a difference in behaviour between the two selection lines when they were exposed to two unfamiliar environments. These results suggest that the behaviour of the HR and LR fish differs when they are challenged in unfamiliar environments, while their behaviour does not differ when they are challenged in their home environment. These observations are in agreement with studies on mammals that show that individuals with reactive coping styles perform similarly to proactive animals when they are challenged in a familiar environment, while they show different behaviour when they are challenged in unfamiliar environments. Thus, these results provide further evidence that the HR and LR selection lines of rainbow trout exemplify the two different coping styles described in mammals.

Keywords

salmonids, behaviour, stress responsiveness, cortisol, coping style, novel object, social interaction, locomotor activity, aggression

Introduction

When an animal is subjected to a challenge that has a negative effect on its fitness this will cause the animal to respond with a combination of behavioural, neuroendocrine and autonomic changes that aim to reduce the adverse effect of that challenge. The change in behaviour allows the animal to either escape or counter the challenge, while the autonomic and neuroendocrine response provides the animal with the resources needed to meet the demands of the altered behaviour as well as maintaining homeostasis during the aversive situation (Moberg, 1985). A fundamental fact is that an identical challenge will produce different behavioural responses among a number of individuals of the same species or indeed within the same population. Numerous studies have shown that these different behavioural traits are distributed in a bimodal fashion along a shy-bold continuum (Koolhaas, et al., 1999). It has also been shown that these traits often are consistent over time as well as across situations (Koolhaas et al., 1999). This implies that these behavioural traits form certain stress response patterns, which are adaptive (Lyons, et al., 1988; Lawrence, et al., 1991; van der Kooij, et al., 2002). So-called "bold" individuals are characterized as more aggressive when confronted with social challenges; they are more active in their attempt to reduce the affect of aversive stimuli and more willing to investigate unfamiliar objects compared to "shy" individuals. In addition to this bold individuals develop routines more easily as a way to deal with different demands, while shy individuals are more flexible in their behaviour. (Huntingford, 1976a; McLeod and Huntingford, 1994; Wilson, et al., 1994; Verbeek, et al., 1996).

Various behavioural studies that have included neuroendocrine parameters have shown that at least two distinct stress response patterns, referred to as proactive and

reactive stress coping styles, exist in mammals (Bohus, et al., 1987; Koolhaas et al., 1999; 2001). The proactive and reactive stress coping style is characterized by behavior patterns that are similar to those described for bold and shy individuals, and these traits are associated with a defined set of neuroendocrine characteristics. Primarily, when exposed to a stressor the proactive individuals display a sympathetic activation (the fight/flight response), while reactive individuals respond with a parasympathetic/hypothalamic activation (the conservation/withdrawal response) (Bohus et al., 1987; Koolhaas et al., 1999). Consequently, the reactive individuals respond to stressors with greater hypothalamic-pituitary-adrenocortical (HPA) axis reactivity, resulting in a larger increase in plasma glucocorticoid levels compared to proactive animals (Koolhaas et al., 1999; 2001).

Although it has not been proved conclusively, there are some studies that suggest that stress coping styles, similar to those observed in other vertebrates, may also be present in teleost fish. For instance, it has been shown that the males of the cichlid, *Nannacara anomala*, display differences in boldness towards a model predator, which correlates with fighting performance (Brick and Jakobsson, 2002). Similarly, it has also been shown that the boldness of three spined sticklebacks (*Gasterosteus aculeatus*) towards a predator correlated with aggressive behaviour shown towards a conspecific (Huntingford, 1976b; 1982) Furthermore, a study on brown trout (*Salmo trutta*) has shown a correlation between the willingness of individuals to inspect a novel object, and the outcome of dyadic fights with size-matched conspecifics (Sundström, et al., 2004). Moreover, there are several studies on rainbow trout that suggest the existence of different stress coping styles similar to those described in mammals. Van Raaij et al. (1996) observed that rainbow trout which displayed strenuous avoidance behaviour when exposed to hypoxia also showed a much larger

catecholamine response compared to the individuals who remained calm during the hypoxia. On the other hand, the calm individuals showed a larger increase in plasma cortisol compared to the individuals that tried to actively avoid this aversive stimulus. In addition to this it has been shown that rainbow trout which display a short latency for the resumption of feeding after a transfer to an environment where they are visually isolated from other individuals also become dominant in dyadic fights with a conspecific which displays a longer latency for the resumption of feeding (Øverli, et al., 2004). However, these studies are unable to conclusively prove the existence of different stress coping styles in teleost fish because they fail to demonstrate a consistency in a divergent behavioural pattern that is associated with a consistent divergence in their physiological stress response. This problem was assessed by Schjolden et al. . This study showed that within a population of juvenile rainbow trout the cortisol response to a confinement stressor is a consistent physiological trait. This study also showed a diversity in behavioural traits that was consistent over time as well as across situations. What this study failed to show was an association between this consistent behavioural stress response and the cortisol response.

The aim of the present study was therefore to establish whether or not rainbow trout divergent in their cortisol response when exposed to a standardized stressor would show a difference in their behavioural response to stress that was consistent across different situations.

Materials and methods

Location and experimental animals

This study was carried out at the fish holding facilities of the Centre for Ecology & Hydrology, Windermere, UK. Elements of this study that required licensing were carried out according to the Animal (Scientific Procedures) Act 1986, and appropriate project and personal licences were in place (TGP and KGTP). The experimental fish were rainbow trout of two F3 lines divergent for cortisol response when exposed to a standardized stressor (confinement). This divergence had been obtained by individual within family selection of fish from the F2 lines as described by Pottinger and Carrick (1999). Prior to the experiments, fish with a high cortisol response (HR) and a low cortisol response (LR) were maintained separately in circular glass fibre outdoor holding tanks (1000 litres), each supplied with 25 l/min flow-through of lake water at ambient temperature. During the experimental period the water temperature varied between 12°C and 14°C. The fish were fed on a commercial diet (Skretting Excel 30 for fingerlings) three times per week at the manufacturers recommended rate. The fish used in this experiment ranged from 20.2 to 40.3 g in weight (mean 29.9 ± 4.2 g, n=40) and from 12.9 to 15.5 cm in length (mean 14.1 ± 0.5 cm, n=40).

Experimental conditions

Home aquaria. These consisted of 10 glass aquaria (90 cm in length, 30 cm in width, water level of 32 cm). Each aquarium was divided into four compartments of equal size (22 cm x 30 cm x 32 cm) by partitions made of grey PVC. The aquaria

were continuously supplied with lake water (1 l/min) at ambient temperature. Light was provided by fluorescent tubes in the ceiling with a light/dark regime of 12/12 hours. This tank system was used for the intruder test and the novel object test. These experiments are described in detail under Experimental protocol.

Stream channel. This consisted of a glass fibre channel divided into two equal parts (each 7.0 m long, 36 cm wide). The water level in the channels increased from 20 cm at the top of the channel (inflow) to 25 cm at the outflow. The channels were continuously supplied with lake water (10 l/min) at ambient temperature. An oval cage of plastic netting without a roof was placed at the end of each channel. Both cages were 36 cm wide and 22 cm across and had a door, which could be remotely opened. At a point 4.3 metres upstream from the cage a shelter had been made out of two piles of stones (approximately 10 cm x 7 cm) with a big flat stone positioned across them (approximately 20 cm x 30 cm). Along the entire length of the channels a screen was erected in order to be able to observe the fish without influencing their behaviour.

Open field aquaria. These consisted of four identical aquaria (60 cm x 30 cm with a water level of 34 cm). Light was provided by four fluorescent tubes (100 W), which were placed behind the aquaria. The walls of the aquaria, except for the one facing forward, were covered in white paper in order to create an evenly lit background. The aquaria were continuously supplied with lake water (1 l/min) at ambient temperature.

Confinement boxes. These consisted of four rectangular black polypropylene boxes (17 cm x 11 cm with a water level of 3 cm) continuously supplied with lake water (0.5 l/min). Each box had a lid, which could be fitted tightly and had a small hole where an anaesthetic solution could be administered.

Experimental protocol

All fish in this experiment were subjected to four different behavioural tests. During the recovery periods between the behavioural tests (3 days) the fish were fed to satiation or a maximum of 0.5 % of their bodyweight each day. All fish did not resume feeding immediately after the tests, but by the end of the recovery period all fish were accepting food again. This was used as an indication that the fish had more or less fully recovered from the previous behavioural test before the next one was conducted.

Intruder test. Twenty fish were randomly selected from each F3 line (HR and LR) and individually isolated in the 40 compartments in the "home aquaria". The fish were then allowed to acclimate for two weeks prior to the experiments. During this period the fish were fed a ration of approximately 1% of their bodyweight each day. After the acclimation period a conspecific, approximately 50% of the bodyweight of the resident fish, was introduced to each compartment. These intruder fish ranged from 9.9 to 19.0 g in weight (mean 15.4 ± 2.2 g) and from 10.2 to 12.5 cm in length (mean 11.5 ± 0.6 cm). All intruders came from the HR line and were naïve to this treatment. During the experiment the behaviour of the pairs of fish was recorded on video. From the video recordings the latency to the first attack by the resident fish was measured. After the first attack the number of aggressive acts (defined as Total Number of Attacks; TNoA) performed by the resident fish during six consecutive five minute periods, were counted. The fish that did not perform any aggressive acts during the first 30 minutes were assigned attack latencies of 1800 seconds and a TNoA equal to zero. Intruder fish were immediately killed after the test was finished.

Risk test. After the intruder test the resident fish were allowed three days of recovery before they were exposed to the next experimental environment; the "stream channel". Individual fish were transferred to the cage at the end of each channel, and left there to settle for 10 minutes. After this period the remotely operated door was opened, giving the fish a free passage to the proper shelter further up the stream. From behind the screens two observers measured the time the fish spent in the cage before swimming out (escape latency). The fish were given a maximum of 30 minutes to leave the cage, and fish that did not leave were assigned an escape latency of 1800 seconds. After this the fish were returned to the "home aquaria" and left to recover for three days before the next experiment.

The open field and novel object test. In this experiment the fish were individually transferred to the "open field aquaria", and their behaviour were recorded on video during 12 minutes immediately succeeding the transfer. After this period the fish were returned to their respective compartments in the "home aquaria". From the video recordings the distance swum by each fish was calculated for 6 consecutive 2-minute periods by the PC program Etho-Vision 3.0 (Noldus Information Technology by The Netherlands). After the open field test the fish were again left to recover for three days before the next experiment; the novel object test. This test was performed in the "home aquaria". Before the fish experienced any novel object, their "basic movement" was recorded on video for 5 minutes. After this the novel object was introduced to each compartment whereupon their movement was recorded for 3 consecutive 5-minute periods. From the video recordings the time spent moving was measured and calculated as percent of total time (5 minutes).

Confinement test. On completion of the open field test the fish were left undisturbed in the "home aquaria" for three days prior to being exposed to a

confinement stressor. For the confinement test the fish were individually transferred to the "confinement boxes". Transfers were staggered to allow time for sampling. The fish were held in the confinement boxes for 1 hour before they were anaesthetised by adding a solution of 2-phenoxyethanol (1:2000) to the boxes. Subsequently a blood sample (approximately 0.2 ml) was collected from the caudal vessels using a syringe pre-treated with Ethylenediaminetetraacetic acid (EDTA). The blood samples collected were immediately centrifuged (13000 rpm, 4°C, 5 minutes) to separate the blood cells from the blood plasma, and plasma were then frozen in liquid nitrogen. These samples were stored at –70°C until required for the analyses of plasma cortisol. After the samples were collected the fish were killed by a blow to the head followed by decapitation.

The concentration of cortisol in the plasma samples was analysed in ethyl acetate extracts using the radioimmunoassay described by Pottinger & Carrick (2001). The antibodies used in this assay are IgG-F-2 and IgG Corp. in a 1:600 proportion. The sensitivity (minimal detection limit) of this assay is 0.3 ng/ml.

Statistical analyses

All physiological and behavioural data are presented as means ± SEM, while the length and weight of the fish are presented as means ± SD. A one-way ANOVA with a Tukey post hoc test was used to test if there were any statistical differences in cortisol response, escape latency and attack latency between the two selection lines.

All statistical calculations were carried out using SYSTAT 8.0 (SPSS, 1998). A two-way repeated measures ANOVA was used to investigate if there was a significant difference in activity (dependent factor) in the open field (N = 6) and the novel object

(N = 4) experiments as well as for the number of attacks (dependant factor) in the intruder test (N = 6) between the two selection lines (time as the within- and selection line as the between subjects factor). These three dependant factors were also tested within each selection line with a one-way repeated measures ANOVA with a single factor to investigate if there was an effect of sampling time (independent factor). The same test was used to investigate if there was a difference between the two selection lines at each time point.

The data on attack latency and total number of attacks were not normally distributed, and therefore the correlation between attack latency and total number of attacks was tested with a Spearman rank order correlation (Sr). The significance of the correlation was tested with Bonferroni probabilities.

Results

Intruder test. Almost all resident fish performed aggressive acts towards the intruder fish within the first 30 minutes of the dyadic interaction. Only 3 individuals from the LR line and 1 individual from the HR line failed to initiate any aggressive behaviour during this period. None of the intruder fish performed any aggressive acts during the experiment. There was no significant [$F_{0.05\ (1,38)}$ = 0.554, P = 0.461] difference between the mean attack latency for this period between fish from the HR line [490 ± 110 seconds, N = 20] and LR line (619 ± 134 seconds, N = 20). However, during the 30 minutes of observation after the first attack fish from the HR line showed a significantly [$F_{0.05\ (5,90)}$ = 8.202, P = 0.010] greater number of attacks compared to the LR line. When comparing the 5-minute intervals the HR line showed a greater number of attacks during the second and third intervals [$F_{0.05\ (1,19)}$ = 7.450, P

= 0.013 and $F_{0.05\ (1,19)}$ = 5.530, P = 0.030 respectively] (Figure 1). Overall, for both lines combined, the number of attacks within each five-minute interval changed significantly during the experiment [$F_{0.05\ (5,90)}$ = 2.931, P = 0.017]. The HR fish reached the maximum number of attacks sooner compared to the LR fish (the 10-15 and 15-20 minute interval respectively) and for both selection lines, there was a steady decline in the number of attacks after these time points (Figure 1). When both HR and LR fish are taken into account there was a significant correlation [Sr = -0.374, P = 0.018, N = 40] between the attack latency and the total number of attacks during this experiment (Figure 2). In this experiment the difference in size between the resident and intruder fish varied. The weight of the resident fish divided by the weight of the intruder fish varied between 1.2 and 3.4 (mean 2.0 ± 0.4). This difference did not significantly affect the attack latency [Sr = 0.136, P = 0.401], but there was a significant correlation with the total number of attacks [Sr = -0.318, P = 0.046] performed in the intruder test. Fewer attacks were performed when the size difference was small.

Risk test. This test provided the fish with a choice of remaining in their starting position, or moving through an area offering no cover to reach a more satisfactory shelter than the one they already occupied. The fish that left the cage during this test either swum away to take refuge underneath the shelter provided upstream or they turned to seek shelter between the channel wall and the outside of the cage. In either case the fish were then occupying an area, which provided better shelter, compared to the inside of the cage. Fish from the LR line remained inside the cage for longer (1532 ± 127 seconds) than fish from the HR line (899 ± 191 seconds). This difference in escape latency between the two selection lines was significant [$F_{0.05\ (1,38)}$ = 7.638, P = 0.009].

Open field test. A significant interaction between time and selection line [$F_{0.05\ (5,90)} = 5.521$, $P < 0.001$; two-way ANOVA with two repeated measures factor] was resolved as a significant difference between HR and LR fish in distance travelled at the first and the last time interval [$F_{0.05\ (1,18)} = 6.205$, $P = 0.023$ and $F_{0.05\ (1,18)} = 8.558$, $P = 0.009$ respectively]. This was most pronounced during the first 2 minutes of the open field test during which the fish from the LR line swam 563 ± 96 cm ($N = 20$) compared to 312 ± 53 cm ($N = 19$) for fish from the HR line (Figure 3).

Novel object test. When fish from the two selection lines were exposed to a novel object, their activity decreased during the first 5 minutes. During the next 10 minutes the activity increased again but did not reach the same level as before the introduction of the novel object (Figure 4). This change in activity was significant [$F_{0.05\ (3,54)} = 4.378$, $P = 0.008$]. There was no significant difference in activity between the fish from the two selection lines [$F_{0.05\ (1,18)} = 0.999$, $P = 0.331$].

Confinement test. After completion of the behavioural experiments, the fish were subjected to a 1 hour confinement stressor. Plasma cortisol levels in the LR fish (32.7 ± 3.0 ng/ml; n = 20) were significantly [$F_{0.05\ (1,36)} = 83.575$, $P < 0.001$] lower than levels in the HR fish (73,7 ± 3.1 ng/ml; n = 20).

Discussion

Stress responsiveness

The rainbow trout that were employed in these studies were from the F3 generation of two lines of fish originally selected for high- and low-responsiveness of plasma cortisol to a confinement stressor. In the present study, when subjected to a 1 hour confinement stressor, fish from the two lines exhibited a markedly divergent plasma cortisol response. This finding was consistent with previous studies on the F2 generation (Pottinger and Carrick, 2001; Trenzado, et al., 2003) and indicated that the behavioural comparisons carried out during the present study had indeed contrasted two groups of fish with a pronounced difference in HPI-axis reactivity.

Behaviour. The results of this study further extend previous results indicating that there are differences in behaviour between the HR and LR lines of rainbow trout (Pottinger and Carrick, 2001; Øverli, et al., 2002). In brief, the swimming activity of the LR fish was significantly greater than that of the HR fish immediately after they were transferred to the "open field aquaria". In addition, the LR fish spent a longer period of time within the cage before exiting into the "stream channel". Finally, HR individuals attacked the intruder more frequently that the LR individuals did during the "intruder test". In contrast to these findings, in some respects the behaviour of the two lines was not different. The attack latency towards the intruders during the "intruder test" was not significantly different between the two lines and they did not differ in their reaction to a novel object. This study therefore suggests that these two lines of rainbow trout sometimes are divergent in their behavioural response to an aversive stimulus, and sometimes they are not. The results of each test will be

considered in more detail and the evidence supporting the parallels between the HR and LR lines, and proactive and reactive coping styles in mammals will be discussed.

Aggression. Our results show that the mean attack latency among the LR individuals was higher compared to the HR individuals. This difference was not significant, but when both selection lines were taken into consideration there was a significant correlation between the attack latency and the total number of attacks during the intruder test. The longer the attack latency the fewer were the attacks during the subsequent 30 minutes of interaction. This suggests that the HR fish were more aggressive than the LR fish. This is not in agreement with previous studies on the HR and LR lines of rainbow trout where it has been shown that LR individuals become dominant when they are allowed to interact in pairs with HR individuals (Pottinger and Carrick, 2001). This suggests that LR fish are more aggressive than the HR fish. Moreover, Höglund et al. (2001), showed that individuals of Arctic charr (*Salvelinus alpinus*) with high levels of aggression, as measured in resident-intruder tests, became dominant after dyadic fights with size matched individuals with lower aggression. It therefore seems contradictory that the HR fish in our study exhibited more aggression during the intruder test than the LR fish. Numerous studies have also shown that those individuals that respond to stress with high HPA-axis reactivity (the mammalian equivalent to the HPI-axis in fish) are less aggressive than those that respond with lower HPA-axis reactivity (Koolhaas et al., 1999). The apparent paradox of our findings may arise because the aggressiveness of the animals in the present study cannot accurately be determined by quantifying the number of attacks towards the intruder. The reason for this is that the interaction between the resident fish and the intruder is a fight for dominance. In this case the outcome of the fight was predetermined because of the substantially lesser size of the intruder, and after the

dominance is achieved by the resident fish the aggressive acts will decrease in numbers (Winberg and Lepage, 1998). Therefore, if the LR fish in our study became dominant in a shorter period of time than was the case for the HR fish, this might account for the fewer attacks performed by the LR fish towards the intruder during the 30-minute period. We do not know if the LR fish became dominant in a shorter period of time compared to the HR fish. Either way dominant status will affect the number of aggressive acts as aggressive acts will affect the status of dominance. Since our data most likely includes observations made after the resident fish had become dominant, the difference in number of attacks in our study cannot be regarded as a quantification of the aggressive capacity of the experimental fish. On the other hand it is likely that the first attack towards the intruder was performed before the resident fish became dominant. The attack latency is therefore a much better indicator of the aggressive capacity of the experimental fish. On the basis of these observations there is nothing to suggest that there is a difference in coping strategy between the HR and LR fish.

Novel object. When a novel object was introduced into the "home aquaria" we observed a reduction in the time spent moving by fish of both selection lines. This is in agreement with numerous studies that have shown that rodents which are exposed to a stressor will reduce the intensity of the behaviour they are performing at the time when an aversive stimulus is introduced (Kudryavtseva, et al., 1991; Koolhaas, et al., 1997; Berton, et al., 1998). This tells us that the introduction of the novel object in this study had an effect on the fish, although it must be characterized as a low or medium intensity stimulus. However, the time spent moving did not differ between the two selection lines at any time interval. This observation suggests that there is no difference in coping strategy between the HR and LR fish.

Open Field test. This test showed that the LR fish were much more active during the first two minutes after the transfer to the open field aquaria. After this period they decreased their activity to the same level as the HR fish. It is commonly agreed upon that proactive coping individuals react to stress with higher activity compared to reactive coping animals (see: Koolhaas et al., 1999). Therefore, the differences in behaviour of the HR and LR fish within this test system suggests that the LR fish exhibit a proactive coping style while the HR fish exhibit a reactive style of coping. It is also worth mentioning that the activity of the LR fish decreased to a level significantly lower than the HR fish during the last time interval. This means that the LR fish changed its behaviour to a larger extent than the HR fish when their initial behavioural response did not reduce or eliminate the stress. This could be a behavioural trait that is important in describing the proactive and reactive coping styles of animals. The consequence of this is that the reactive animals is not always more flexible in their behaviour than proactive animals.

Stream channel. When the fish left the cage situated the end of the stream channel they immediately sought a refuge that could be regarded as superior to the starting cage, offering a greater degree of cover. The fish were not observed to engage in exploratory behaviour and therefore we interpret their actions as indicating a desire to leave an environment they considered unsatisfactory in some way. However, there were a lot of individuals (more than half) that chose to stay within the starting cage for the entire experiment, significantly greater numbers of which were LR fish. This may be interpreted as suggesting that the individuals from the LR line are more stereotypic, and less flexible, in their behaviour compared to the HR individuals. The higher level of flexibility seen within the HR strain is a behavioural trait commonly considered to be characteristic of individuals that exhibit, a reactive coping strategy

while the more stereotypic behaviour shown by the LR line is consistent with the behavioural pattern shown by proactive coping animals.

Coping styles. As mentioned in the introduction, the reactive coping strategy is characterized by flexible behaviour and low levels of aggression. In this regard it has been shown in rodents that heritable stress coping strategies are characterized by individual differences in aggression (Benus, et al., 1991). Moreover, it has also been shown that aggressive individuals show exclusively proactive behaviour as a response to stress, while non-aggressive individuals can respond both reactively and proactively (Benus, et al., 1989). This latter point may offer in part an explanation of why the two different strains of rainbow trout in our study responded similarly to an aversive stimulus and sometimes they responded differently. Moreover, it has been shown that wild house mice that have been genetically selected for long attack latencies would respond proactively to an aversive stimulus if they were exposed to that stimulus in a familiar environment, while they would respond reactively to the same stimulus in an unfamiliar environment (Sluyter, et al., 1996). This study also showed that the short attack latency mice responded proactively regardless of the environment. Our study has also shown that the difference in the behavioural response to an aversive stimulus was apparent between the two selection lines when they were exposed in an unfamiliar environment. On the other hand we found no difference in their behavioural response when they were exposed within in the home aquaria, which will have to be characterized as a familiar environment. These observations are in agreement with studies on mammals that show that individuals with reactive coping styles perform similarly to proactive animals when they are challenged in a familiar environment, while they show different behaviour when they are challenged in unfamiliar environments. This further supports the hypothesis that these HR and LR

strains of rainbow trout represent the two different stress coping styles akin to those described in mammals.

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FIGURE LEGENDS

Fig. 1. The number of attacks performed by the resident LR (black) and HR (white) fish towards the intruder during six consecutive five-minute periods. Significant differences between the LR and HR fish are denoted by asterisks (* denotes a significance level of $P < 0.05$). $N = 20$ for both selection lines.

Fig. 2. The Spearman rank correlation ($R = -0.374$, $P = 0.018$) between the attack latency and the total number of attacks performed by both LR (black) and HR (white) fish. $N = 20$ for both selection lines.

Fig. 3. The distance moved (cm) by LR (black) and HR (white) fish subsequent to the transfer to the open field aquaria. Significant differences between the LR and HR fish are denoted by asterisks (* denotes a significance level of $P < 0.05$; ** denotes a significance level of $P < 0.01$). $N = 20$ for LR fish and $N = 19$ for HR fish.

Fig. 4. The time spent moving (% of total time observed) by LR (black) and HR (white) fish before (Basic Movement; BM) and after (0-15 minutes) the introduction of a novel object into the home aquaria. $N = 20$ for LR fish and $N = 19$ for HR fish.

Figure 1.

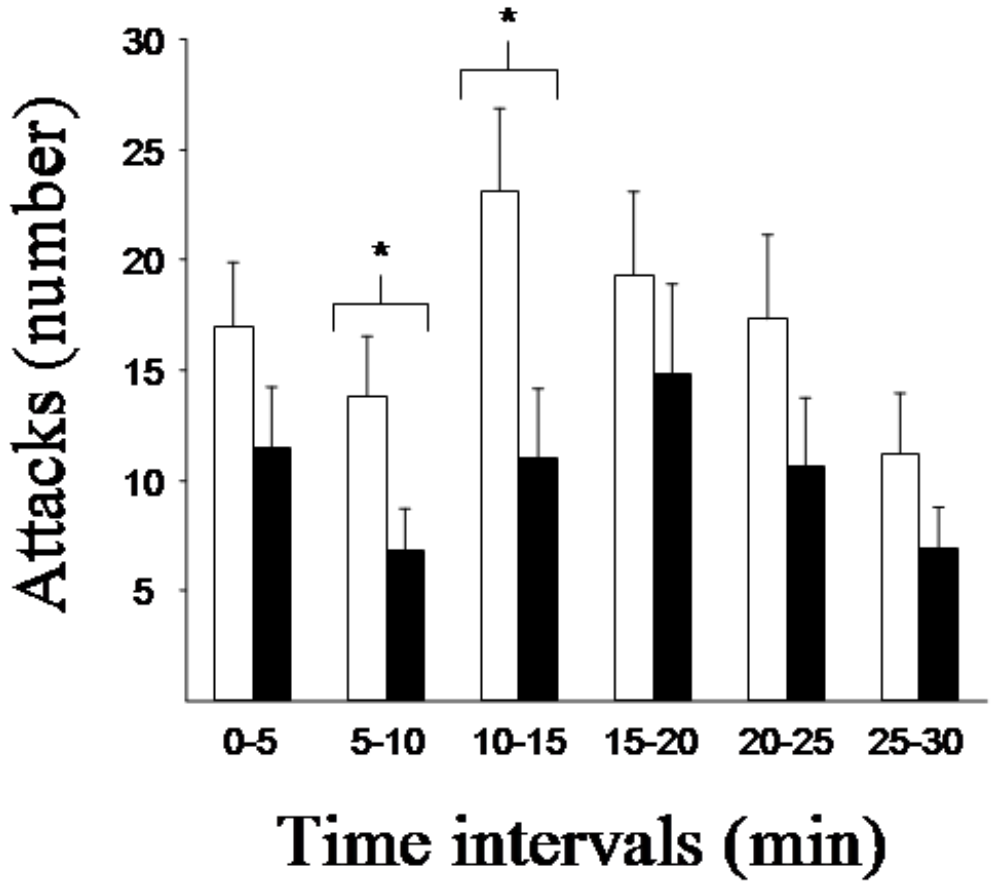
Attacks (number)
30
25
20
15
10
5
*
*
0-5
5-10
10-15
15-20
20-25
25-30
Time intervals (min)

Figure 2.

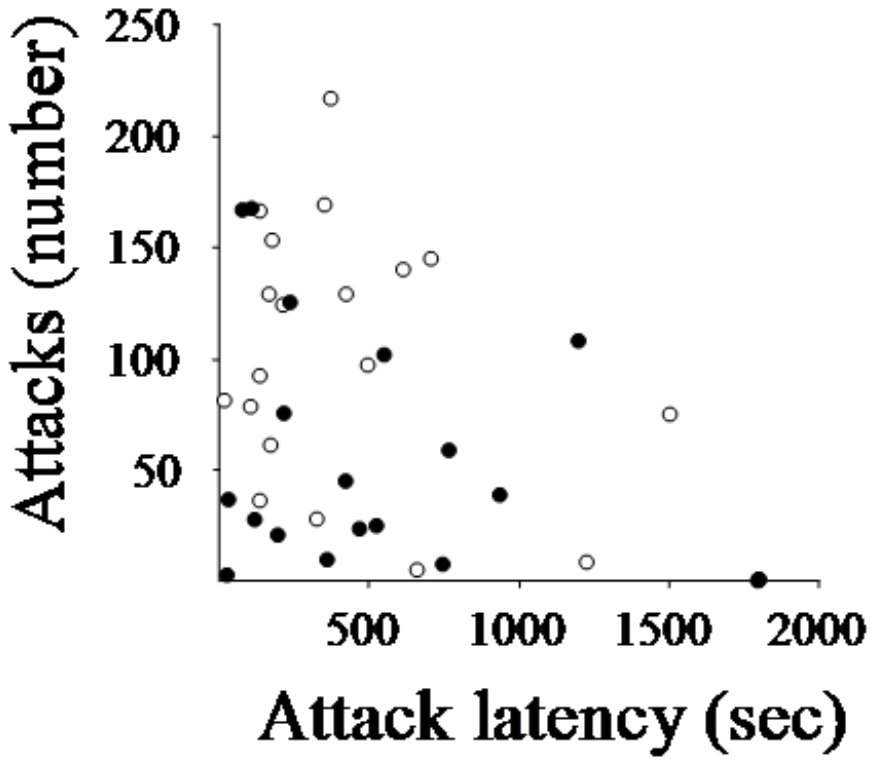
Attacks (number)
250
200
150
100
50
500
1000
1500
2000
Attack latency (sec)

Figure 3.

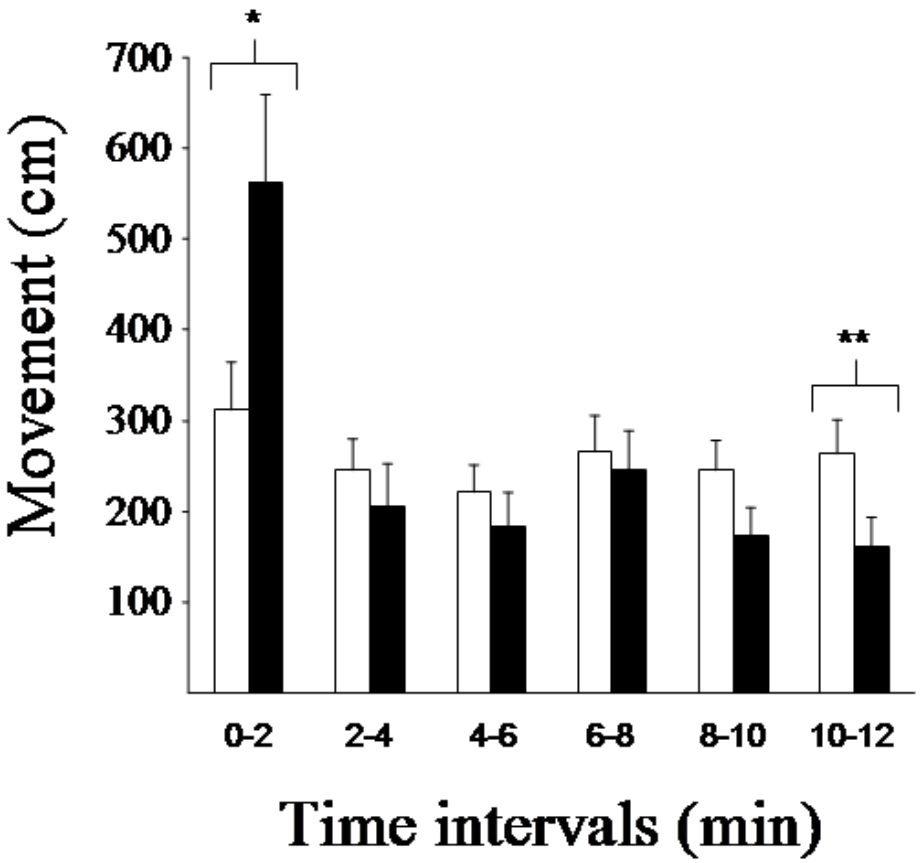
*
**
700
600
500
400
300
200
100
Movement (cm)
0-2
2-4
4-6
6-8
8-10
10-12
Time intervals (min)

Figure 4