Behavior Genetics
July 2014, Volume 44, Issue 4, pp 383-393
http://dx.doi.org/10.1007/s10519-014-9652-z

Assessment of Genetic Variability of Fish Personality Traits using Rainbow Trout Isogenic Lines

Sandie Millot[1, *], Samuel Péan[1], Laurent Labbé[2], Thierry Kerneis[2], Edwige Quillet[3], Mathilde Dupont-Nivet[3], Marie-Laure Bégout[1]

[1] Laboratoire Ressources Halieutiques, Ifremer, Place Gaby Coll, BP 7, 17137, L'Houmeau, France
[2] INRA, UE0937 PEIMA Pisciculture Expérimentale INRA des Monts d'Arrée, Le Drennec, 29450, SIZUN, France
[3] INRA, UMR 1313, GABI Génétique Animale et Biologie Intégrative, F-78350, Jouy-en-Josas, France

*: Corresponding author : Sandie Millot, email address : sandiemillot@yahoo.fr

Abstract:

The study of inter-individual variability of personality in fish is a growing field of interest but the genetic basis of this complex trait is still poorly investigated due to the difficulty in controlling fish genetic origin and life history. When available, isogenic lines that allow performing independent tests on different individuals having identical genotype constitute a very relevant experimental material to disentangle the genetic and environmental components of behavioural individuality. We took advantage of heterozygous isogenic lines to investigate the personality in rainbow trout through the analysis of their reactions to different experimental situations. To this end, seven to ten rainbow trout isogenic lines were screened for their spatial exploratory behaviour, their flight response toward a stressor and their risk taking behaviour. Results showed that some lines seemed less sensitive to new events or environmental changes and could be defined as low responsive, while others were very sensitive and defined as high responsive. The use of isogenic lines highlighted the importance of genetic factors, in combination with life history, in the expression of personality in domesticated fish.

Keywords: Personality ; Isogenic lines ; Genetic variability ; Risk taking ; Spatial exploration ; Oncorhynchus mykiss

1. Introduction

Many studies in animals have reported that individuals, though similar for many characteristics (age, size, sex, maturity stage) may differ markedly in their behaviour pattern (Slater, 1981; Martin, 1991). These individual behavioural differences, consistent over time and across situations, are usually defined as temperament (Réale et al, 2007), personality (Bell, 2005), coping style (Koolhaas et al., 1999) and all together form behavioural syndrome (Sih et al, 2004). Generally speaking, proactive individuals are characterized by a fight or flight reaction including: active avoidance, aggression, high sympathetic reactivity but only modest elevations of plasma cortisol levels. Moreover they have a tendency to follow and develop behavioral routines. Reactive individuals respond to challenges with a "freezing" behavior and a more pronounced cortisol release. But in contrast to proactive, they show flexibility in their behavioral responses (Koolhaas et al., 1999; Dingemanse et al., 2010).

There is accumulating evidence that behavioural syndromes are not restricted to higher vertebrates (Riechert and Hedrik, 1993; Bell and Stamps, 2004; Aragon et al., 2006), that they often have underlying neuroendocrine and also physiological correlates as mentioned above (Koolhass et al., 1999; Feldker et al., 2003; Øverli et al., 2006) and heritable genetic variation in behavior of fish has been demonstrated (reviewed in van Oers et al., 2005, and exemplified recently in farmed Atlantic cod by Drangsholt et al. 2014). The study of personality differences has proven many utilities such as improvements in animal production, welfare and enhanced knowledge in conservation ecology (Boissy and Bouissou, 1995, Le Neindre et al., 1996, Cote et al. 2010). The genetic basis of personality in fish has however been poorly investigated due to the difficulty in controlling fish genetic origin and life history.

In aquaculture, selective breeding of fish that would present minimal responsiveness to husbandry practices is one of the strategies considered to improve fish welfare. Thus, the interest in stress-tolerant strains better able to cope with the unavoidable stressors inherent to aquaculture has increased over the past two decades (Pickering, 1992, 1993; Overli et al., 2006; Pottinger, 2006; Weber and Silverstein, 2007). But more importantly, a well adapted animal to husbandry environment is not only a fish which will produce less stress hormones, but also an animal able to express a coherent and appropriate set of behavioural responses according to the nature of the stressor, such as a fast exploration behaviour when placed in a new rearing tank to find quickly the new food source. At the moment, experimental selections for stress-resistance have focused mainly on physiological traits such as post-stress levels of cortisol (Fevolden and Røed, 1993; Fevolden et al., 1991; Pottinger and Carrick 1999; Weber et al., 2008; Rexroad et al., 2012) and to a lesser extent, lysozyme (a stress-labile component of the non-specific immune system suggested to be an indicator of the degree of stress-caused physiological disturbances; Fevolden et al., 1993; Weber et al., 2008).

In rainbow trout (*Oncorhynchus mykiss*) it has been shown that the magnitude of the cortisol response to a standardised confinement stressor is an individual's characteristic which has a moderate to high degree of heritability (Pottinger and Carrick, 1999; Weber et al., 2008; Rexroad et al., 2012) and several genomic regions responsible for the variability of the trait have been identified (Drew et al., 2007; Rexroad et al., 2012, 2013; Quillet et al., in press). Lines of high- (HR) and low-responsive (LR) trout have been selected on the basis of their cortisol response to stress (reviewed by: Øverli et al., 2005, 2007) and were used as a model system over a number of generations to study behavioural differences among individuals and the interactions between the neuroendocrine stress mechanisms and behaviour. A range of consistent differences (*e.g.* time for feeding or swimming recovery, social status) have been

documented between the HR and LR lines (reviewed by: Øverli et al., 2005, 2007). However, to go further in the understanding of the origin and the significance of such individual behavioural differences, it is important to improve our knowledge on the genetic basis of personality traits and interactions with environmental conditions.

The use of isogenic lines, thanks to their genetic uniformity, is a considerable advantage for the simultaneous examination of genetic and environmental effects on behavioural individuality (Vrijenhoek, 1994). Indeed, isogenic lines make it possible to avoid interference of learning effect when performing repeated observations of the "same genetic individual" (the line genotype), which considerably improves the precision of the individual behavioural phenotype characterisation. It is also a real advantage to study individual characteristics which are potentially influenced by environment and life history. Though only few studies are available in fish due to the difficulty to implement isogenic lines, such lines were already successfully used to analyse behavioural traits in salmonids (Iguchi et al. 2001, Lucas et al. 2004).

The purpose of the present study was thus to investigate the genetic variability of behavioural individuality through the analysis of fish reactions to different experimental situations. To this end, seven to ten rainbow trout heterozygous isogenic lines were screened using three types of behavioural tests that were previously used successfully in sea bass (*Dicentrarchus labrax*) to analyse the spatial exploratory behaviour and flight response toward a stressor (Millot et al., 2009a) and to evaluate the risk taking behaviour (Millot et al., 2009b). The main aim of this study was to determine relevant behavioural indicators, in this case applied to discriminate behavioural responses observed in domesticated heterozygous isogenic lines, and able to direct and focus research in identifying genotypes well adapted to farming conditions.

2. Material and methods

This study was conducted under the approval of the Animal Care Committee of France under the official licence of M.L. Bégout (17-010).

2.1. Experimental fish

Experimental fish were produced in the INRA experimental farm (PEIMA, Sizun, France). Broodstock were issued from the INRA homozygous isogenic rainbow trout lines maintained at PEIMA. The lines were previously established after two generations of gynogenesis and further maintained by within line single pair mating using sex reversed XX males (Quillet et al., 2007). Within each line, all fish are homozygous and genetically identical, *i.e.* constitute replicates of the same genotype. Founder females at the origin of the homozygous lines were randomly sampled from the INRA 'synthetic' strain (SY), a domesticated population expected to contain a large amount of genetic variability.

The ten heterozygous isogenic lines used in the experiment were obtained by mating homozygous females from one single isogenic line with ten individual homozygous XX-males from 10 other isogenic lines. To get enough eggs for the whole experiment, ova were collected from several females of the maternal isogenic line. The use of a single maternal line (*i.e.* same genotype) aimed at minimizing initial maternal effects associated with egg size and hatching time. Moreover, to further minimize maternal effects, the 24 dams used were chosen among 74 spawns collected on the same day to get spawns with similar mean egg size (ranging from 30.7 to 31.9 mg). Eggs were carefully mixed and then divided into ten groups, each one being fertilized by milt from one of the ten sires. Because of the homozygozity of both sire and dam, each of the ten progeny contains genetically identical heterozygous individuals leading to no disruption from normal genotype / environment interactions. Since there is only one maternal line, only genetic effects (paternal genetic contribution) differ

among progeny. Homozygosity of all breeders and isogenicity of dams were checked using allelic variation at four polymorphic microsatellite markers for dams and nine markers for sires.

Fertilized eggs were incubated at 11.4°C. At eyed stage, each progeny was divided into three replicates that were reared in a 0.25 m^3 tank each using natural spring water (11.4°C) until 158 days post fertilization (dpf). Every replicate was then transferred into a 5.4 m^3 tank supplied with river water (11.3-16°C).

At 198 dpf, 50 fish from each tank (*i.e.* 150 fish per line; mean weight: 39.6 ± 7.9 g) were randomly sampled and individually PIT (passive integrated transponder)-tagged. After anaesthesia, tags (12 mm long x 2 mm in diameter) were inserted horizontally in the dorsal muscle just behind the head. The tagged fish were redistributed into three 6 m^3 tanks (3 m diameter × 1 m deep) of 500 fish (10 lines, 50 individuals per line) supplied with river water. Those fish were used for the risk taking behaviour tests that were realized between 347-363 dpf (mean weight: 199.2 ± 42.6 g). The water temperature fluctuated between 5 and 11°C, oxygenation was always above 90% of saturation in the water outlet, and fish were submitted to the natural light regime (8L:15D) and fed by self-feeders (Boujard et al., 2002; Millot et al., 2008) with a commercial diet for rainbow trout (3 mm organic food, Le Gouessant, France).

At 304 dpf (mean weight: 157.3 ± 50.2 g) 140 tagged fish (20 individuals x 7 lines) were transferred to Ifremer facilities (La Rochelle, France) for the spatial exploratory behaviour and flight response tests. Fish were kept in mixed groups in 5 tanks (400 l) in dechlorinated and filtered tap water and hand fed the same commercial diet as previously. The water temperature was maintained at 16 ± 1.5°C and oxygenation above 90% saturation in the outlet both during their acclimation time (for 70 days) and during the assays (Experiment 1, see below).

2.2. Experimental set up and procedure

2.2.1. Spatial exploratory behaviour and flight response; Experiment 1

The experiment was performed in the facility of Ifremer La Rochelle, in a room dedicated to fish behaviour study. The experiment was carried out in a 400 l tank similar to the fish home tank and with the same water conditions. The experimental tank was sheltered by black curtains and lit with one spotlight located to minimise shadow. The stimulus was a tube full of sand of 67 g, 96 mm length and 25 mm diameter, which fall was driven by gravity after an electromagnet (which kept the stimulus suspended) was inactivated. An opaque pipe (1.6 m length, 35 mm diameter) was fixed 2 cm above the water surface to hide the stimulus during its fall and to allow recording the fish reaction at the moment of impact. A Mini color CMOS camera (Velleman) was located at 1.6 m above the water surface and video was recorded on a hard disk recorder (see. Fig. 1A in Millot et al., 2009a).

The experiment was realized on 10 fish per line (between 374 – 444 dpf, mean weight: 253.6 ± 53.2 g; Table I); 7 lines were screened. A single fish was quickly moved from its home tank to the experimental tank for two hours of acclimatization. Even if the procedure to place fish in the experimental tank involved stress, it was standardized for each fish and thus allowed to evaluate the swimming responses for each individual in the same way. After the acclimatization time, the video was recorded and stimulation occurred after 30 min or until fish moved in all directions in the entire tank. The stimulus was dropped by inactivating the electromagnet when the fish reached the stimulation zone (i.e. zone 1; see Fig. 1B in Millot et al., 2009a) and fish behaviour was continuously recorded during one hour after the stimulation (see Fig. 2 in Millot et al. 2009a). After the test, fish were removed from the experimental tank, measured for weight and length and placed in a separate holding tank to avoid alarm pheromone release within the fish group which remained to be tested. The test was run only once per individual.

2.2.2. Risk taking behaviour; Experiment 2

The experiment was performed in the outside facility of the PEIMA. The experiment was carried out in a fourth tank of 6 m³ similar to the ones used to maintain the experimental fish. To monitor the risk-taking behaviour, the experimental tank was separated in two equal zones (safe *vs.* risky) by an opaque divider. The safe zone was shaded and gathered all fish at the beginning of the experiment. The risky zone was naturally lit and included the self-feeder (not working during the test) and the usual feeding area. The opaque divider had a circular (12 cm diameter) opening in its centre that was equipped with a PIT-tag detection antenna connected to a control device and a computer. Such a set up allowed the individual passages through the opaque divider to be monitored with the associated time.

The experiment was performed on the three replicates of 500 fish (50 fish per line x 10 lines) between 347-363 dpf (mean weight: 199.2 ± 42.6 g; Table I). Two consecutive tests 16 days apart (T1, T2) were done on the fish groups (T1: 347 dpf and T2: 363 dpf) and according to the same procedure, each test lasting 23 hours. The fish were transferred to the experimental tank 24 hours before the test started. During this recovery time, the opening in the partition was blocked and the test started at 12:00 on the next day when the opening was freed.

2.4 Data analysis

2.4.1. Experiment 1

The video recordings were analysed using the software EthoVision XT (Noldus, The Netherlands), which allowed to separate the tank in four virtual zones of equal surface: zone 1 containing the stimulation zone, zone 2, zone 3 and zone 4 further away from the stimulation zone and having most edges in contact with tank walls (protective area); see Fig. 1B in Millot et al., 2009a) and to track the fish swimming behaviour.

Each video recording were analysed in three sequences of 20 min: sequence 1 (S1): before the stimulation; sequence 2 (S2): just after the stimulation and sequence 3 (S3): 40 min after the stimulation.

Different variables of interest were chosen to analyse the fish behaviour:

- The proportion of time spent by a fish in each zone (residence, *R* in %). This variable allowed identifying the fish spatial distribution for each sequence.

- The distance travelled by each fish in the tank (*D*, in m). This variable quantified the fish swimming activity level in the tank for each sequence.

- The differences in distance travelled between two consecutive sequences were calculated ΔD (in m).

For this experiment, comparison among lines provided information on differential exploration capacities and flight response.

2.4.2. Experiment 2

To assess group behaviour, the percentage of the total fish number per isogenic line entering in the risky zone was calculated.

For each individual, risk-taking behaviour was evaluated by analysing: the total time spent in the risky zone (*Tt*) and the total number of passages through the opening (*Np*). The individual score emergence (*Se*) was also calculated as: $Se = (td - te)\ td^{-1}$, where *td* is the test duration (total test duration was equal to 1380 min) and *te* is the emergence time (the time necessary for the first entry in the risky zone; in min). Score emergence close to 0 therefore corresponded to a very late or no entry in the risky zone, while close to 1 it corresponded to a very fast entry.

The comparison of the data among lines provided information on differential performances to take risk in a given test, and comparison between tests allowed assessing the differential habituation and/or learning capabilities.

2.5. Statistics

All analyses were performed using Statistica 7 software and for all tests, significant threshold was $p < 0.05$.

2.5.1 Experiment 1

All data were analysed for normality with a Shapiro-Wilk test and for homogeneity of variance with a Bartlett's test; they all complied the rules for parametric statistics. For the individual fish spatial distribution, since tank zones were not independent, a two fixed factors ANOVA was used to compare the differences between lines and sequences for zones 1 and 4. A null model of space use was tested: the fish spatial distribution (residence, *R*) was compared to a theoretical homogeneous distribution in zone 1, zone 2, zone 3 and zone 4 (25% in each zone) by a Kolmogorov–Smirnov test. The regression between each swimming variable (*D* and ΔD) and body weight was significant so that an ANCOVA with fish weight as covariate was used to compare the differences between lines and sequences for the fish swimming variables. Parallelism of regression slopes was verified for each variable studied (*i.e.* the slope of the regression line for each variable against fish weight did not differ significantly between fish of different lines). Homogeneous groups of lines were determined with the *a posteriori* Newman and Keuls test (Dagnélie, 1975).

2.5.2. Experiment 2

Data were analysed for normality with a Shapiro–Wilk test and for homogeneity of variance with a Bartlett's test. The variables 'total time spent by a fish in the risky zone (*Tt,* %)' and 'individual score emergence (*Se*)' underwent an arcsine transformation to normalize data (Sokal & Rohlf, 1995). Then, repeated ANOVAs were used to analyse the average differences of the proportion (%) of fish entering in the risky zone and of the total time (*Tt*) spent by fish in the risky zone between lines and tests. The hypothesis of parallelism was verified for each variable studied. This involved that the slope of the regression line for each variable against fish weight did not differ significantly between fish of different lines. ANCOVA (with individual fish weight as covariate) was then used to analyse the average differences of total number of fish passages (*Np*) through the opening and of fish score emergence (*Se*) between lines and tests. Homogeneous groups were determined with the *a posteriori* Newman & Keuls test (Dagnélie, 1975). Pearson correlations between the two tests (T1 and T2) for individual and mean line total time spent in the risky zone, number of passages through the opening and score emergences were calculated as indicators of fish and isogenic line personality consistency over time.

2.5.3. Correlation between experiments

Pearson's coefficients of correlation between mean variables values from each isogenic line measured in Experiment 1 and 2 were calculated. The mean distance travelled (m) during S1, S2 and S3 in Experiment 1 were compared to the three variables measured during the two tests of Experiment 2 (*i.e.* percentage of the population entering in the risky zone (%), time spent in the risky zone (min) and score emergence).

3. Results

3.1. Spatial exploratory behaviour (Experiment 1)

3.1.1. Spatial distribution

The overall mean time spent by fish in zone 1 ($F_{2,189} = 0.24$, $p > 0.05$) and in zone 4 ($F_{2,189} = 0.02$, $p > 0.05$) did not change over time (S1, S2 or S3). However, there were differences in spatial distribution between lines for zone 1 ($F_{6,189} = 5.29$, $p < 0.001$) and for zone 4 ($F_{6,189} = 3.23$, $p < 0.05$) whatever the experimental sequences. Indeed, A03h fish spent less time in zone 1 than fish from the other lines and lines A03h and B45h spent more time in zone 4 than the other lines (Table II). There was no significant interaction between sequence and line ($F_{12,189} = 5.28$, $p > 0.05$). Whatever the sequence, the overall proportions of time spent by fish in zone 1 (20 ± 2%), zone 2 (34 ± 3%) and zone 3 (37 ± 3%) were not significantly different from the expected ones under the hypothesis of a theoretical homogeneous spatial distribution among zones (25%). However, the proportion of time spent in zone 4 was always significantly lower than expected (9 ± 2 %; d= 0.49, $p < 0.01$ for S1; d= 0.43, $p < 0.05$ for S2 and d= 0.44, $p < 0.05$ for S3).

3.1.2. Swimming activity

For all lines, fish travelled more distance during the first sequence than during the two following sequences S2 and S3 ($F_{2,188} = 5.28$, $p < 0.01$; Fig. 1), while the distance travelled by fish for each line remained stable between S2 and S3. Whatever the sequence, there was significant difference among lines ($F_{6,188} = 6.42$, $p < 0.001$) with A03h fish travelling less distance than other lines, contrary to A02h fish that were the most active.

There was interaction between lines and experimental sequences also for the magnitude of the change in distance travelled (ΔD, $F_{6,125} = 3.44$, $p < 0.01$). Lines B45h and B61h showed the highest decrease between sequences 1 and 2 (-85 ± 20 m and – 82 ± 20 m on average respectively), while line A02h exhibited the lowest decrease (-11 ± 26 m).

3.2. Risk taking behaviour (Experiment 2)

3.2.1. Percentage of the total fish number per isogenic line entering in the risky zone

Overall, the percentage of total fish number entering in the risky zone was higher during the first test (T1) than during the subsequent one (T2; Fig.2; $F_{1,40} = 4.91$; $P < 0.05$) and there were marked differences between lines ($F_{9,40} = 13.86$; $P < 0.001$). There was significant interaction between test and line factors ($F_{9, 40} = 2.21$, $p < 0.05$) but for both tests the proportion of fish entering in the risky zone was always significantly lower for lines A02h, A22h and AP2h than for lines A03h, B61h and B45h.

3.2.2. Total time spent by fish in the risky zone

As a general feature, all fish from all lines spent less time in the risky zone during T2 than during T1 (Fig.3A; $F_{1,2630} = 56.31$; $P < 0.001$). There were differences between lines ($F_{9,2630} = 2.18$; $P < 0.05$) and for both tests the time spent in the risky zone was always lower for lines A02h, A22h and AP2h than for lines A03h, B61h and B45h.

A weak but significant correlation was found at the individual level for the total time spent in the risky zone between both tests ($R = 0.13$, $p < 0.001$, $n = 1325$). Interestingly, the correlation was dramatically increased when the total time spent in the risky zone was averaged by isogenic line ($R = 0.89$, $p < 0.001$, $n = 10$), indicating that mean line behaviour was highly consistent across tests for this trait.

3.2.3. Total number of fish passages through the opening

Whatever the line, the total number of fish passages through the opening was lower during T2 than during T1 (Fig.3B; $F_{1,2627} = 10.37$; $P < 0.01$). There were differences between lines ($F_{9,2627} = 38.47$; $P < 0.001$) and for both tests the total number of passages through the opening was always lower for lines A02h, A22h and AP2h than for lines B61h and B45h.

A weak but significant correlation was found at the individual level for the number of passages through the opening between the two successive tests ($R = 0.16$, $p < 0.001$, $n = 1325$). Again, the correlation was considerably increased when the number of fish passages was averaged by isogenic line ($R = 0.91$, $p < 0.001$, $n = 10$), indicating a high consistency of line (individual genotype) behaviour across tests for this trait.

3.2.4. Score emergence

As a general feature all fish from all lines presented a lower score emergence during T2 than during T1 (Fig.3C; $F_{1,2627} = 177.21$; $P < 0.001$), indicating that fish entered earlier in the risky zone during T1. There were differences between lines ($F_{9,2627} = 23.62$; $P < 0.001$) with lines A02h, A22h and AP2h exhibiting lower score emergence than lines A03h, B61h and B45h whatever the test.

A weak but significant correlation was found between successive individual score emergence between the two tests ($R = 0.20$, $p < 0.001$, $n = 1325$). Once more, the mean line behaviour was very consistent across tests (correlation between the averaged score emergences was $R = 0.92$, $p < 0.001$, $n = 10$).

3.3. Correlation between experiments

There were strong correlations between the isogenic lines performances in Experiment 1 and the test T1 of Experiment 2 (except for the number of passages through the opening). No significant correlations were observed between performances in Experiment 1 and test T2 in Experiment 2.

Indeed, whatever the sequences of the Experiment 1 (S1, S2 and S3) there were strong negative correlations between the mean distance travelled in the tank and the percentage of the population entering in the risky zone, the time spent in this zone and the score emergence

during T1 of Experiment 2 (Fig.4). It revealed that the more an isogenic line was active during Experiment 1, the less risk it took during the first test of Experiment 2. Two extreme isogenic lines could be distinguished: line A03h which exhibited the lowest swimming activity throughout the three sequences of Experiment 1 and the highest risk taking during T1 of Experiment 2, and line A02h which showed the highest swimming activity during the entire Experiment 1 and the lowest risk taking behaviour during T1 of Experiment 2.

4. Discussion

The spatial exploration behaviour is generally considered as a good indicator of animal coping with its environment and usually depends on the individual personality. The first part of Experiment 1 (sequence 1) could be considered as an open field test, since each fish was isolated and placed in a novel environment. The results revealed that even after two hours of acclimatization, there were significant differences among isogenic lines, with two extreme lines reacting oppositely to the situation. Indeed, fish from line A03h showed a low level of swimming activity and appeared highly stressed when placed in isolation in the novel environment (close to tank walls in zone 4) while fish from line A02h showed homogeneous swimming and exploration behaviour through the entire tank. This contrasted behavioural response could be compared to the observations done by Schjolden et al. (2005) on two lines of rainbow trout divergently selected for post-stress cortisol response (high cortisol response -HR- and low cortisol response -LR-). These authors showed that when placed in open field LR individuals presented a higher swimming activity than HR fish. This difference in behaviour however disappeared after 2 min, contrary to our study where line A02h expressed a higher swimming activity than line A03h during the entire period of survey (two hours). Thus the spatial exploration behaviour in a new environment seems to be one of the

components of fish personality dependent on genetic factors (as shown recently by Drangsholt et al. 2014).

In our study, all lines showed a decrease in swimming activity after the stimulation which is very consistent with the observation that freezing, decrease of swimming activity and avoidance of risky area are the most common fish anti-predator behaviours (Giles and Huntingford, 1984; Wisenden et al., 1995; Brown and Smith, 1997). Reduction of fish swimming activity could be also observed when a novel object (*e.g.* the stimulus) is introduced into the aquarium (Schjolden et al., 2005). In our study, lines B45h and B61h seemed particularly affected by the stimulus fall and showed a higher flight response than other fish lines (Sequence 2), which strongly suggests the existence of a genetic control of this trait. This result confirms and extends previous observations using groups with distinct genetic origin in salmonids. Indeed, Iguchi et al. (2001) showed that the freezing time after predator exposure was significantly different between two clones of red-spotted cherry salmon. Lucas et al. (2004) demonstrated that clonal rainbow trout line derived from population with high level of domestication showed shorter duration of startle responses than clonal line derived from more recently domesticated population.

During the last part of the experiment (Sequence 3), fish did not recover their initial swimming activity except for line A02h, which indicated that in most of the lines, fish remained fearful toward the stimulus.

Behavioural differences between lines have also been observed during the risk taking challenge (Experiment 2). The first test (T1) of this experiment showed that whatever the isogenic line, more than 60% of the population passed through the opaque partition to reach the risky zone. This high proportion differs markedly from the one observed with sea bass (Millot et al., 2009b) where only 23% of the population entered in the risky zone during the

first test. This result could be explained by the fact that for trout, the experiment has been performed with a high number of fish (500 per tank, closer to aquaculture condition and allowing to test all isogenic lines in the same time) and the phenomenon of leadership (initiation of a movement made by one or some individuals and followed by the rest of the group; Krause et al. 2000) has probably played a role in determining the high proportion of fish population entering in the risky zone. Another explanation could be the level of domestication (higher for rainbow trout than for sea bass) which tends to reduce predator avoidance and to increase risk taking behaviour in salmonids (Berejikian, 1995; Johnsson and Abrahams, 1991; Johnsson et al., 1996, Lucas et al, 2004). Indeed, as shown by Price (1998, 1999) the absence of predators in captive environments may lead to relaxed selection on, or even selection against, frequent displays of predator avoidance behaviour patterns. This hypothesis could be confirmed by the general lower time spent in the refuge zone (zone 4) in Experiment 1 than expected, highlighting thus the relative weak stress level of this highly domesticated species when placed in a novel environment.

The proportion of fish per isogenic line entering into the risky zone, the number of passages through the partition, the time spent in the risky zone and the emergence score decreased significantly for all lines during the second test. The decrease observed for all variables can be considered as a loss of fish interest for the risky zone or a decrease of their curiosity level. This decrease of interest or curiosity could be explained by the fact that fish learned that the risky zone did not contain any food reward and thus did not trigger any risk taking behaviour. Indeed, Leblond and Reebs, (2006) showed that risk-taking behaviour is usually the result of a trade-off between risk aversion and other motivations such as hunger, curiosity or need to maintain inter-individual distance.

Even if these behaviour changes between the two successive challenges were observed for all isogenic lines, A03h, B61h and B45h showed always higher risk-taking behaviour or curiosity

than lines A02h, A22h and AP2h. This result could be again compared to the observations of Schjolden et al. (2005) who showed that LR fish spent a longer period of time within the cage before exiting into the "stream channel" which could be assimilated to the risky zone in the present study. This result suggests that individuals from LR line or in our case from lines A02h, A22h and AP2h may be more stereotypic and less flexible in their behaviour compared to the HR or A03h, B61h and B45h lines.

This study revealed also a strong behavioural consistency of each isogenic line (*i.e.* each individual genotype) during the two subsequent tests, which highlighted that risk taking behaviour or curiosity, is highly influenced by genetic factors. This result is in accordance with the studies of Iguchi et al. (2001) and Lucas et al. (2004) who evidenced differences in behavioural patterns among two cherry salmon clonal lines. However, the present study revealed that there is still within line individual variability in the response to successive tests. Indeed, the correlations between time spent in the risky zone, score emergence and number of passages during T1 and T2 are much higher when considering the mean line value than considering the individual values. This variability within line could be due to both the individual life history (*e.g.* food intake, social status) and the responsiveness to environmental clues (*e.g.* food delivery: time, place or husbandry stressors: light, noise).

Wolf et al. (2008) suggest also that the existence and consistency of individual differences in response to challenge could be explained simply by the extent to which each individual perceive and react to environment stimuli. According to this model, some individuals are highly sensitive to environmental changes and readily modify their behaviour according to the prevailing conditions (high responsive and flexible). In contrast, others pay little attention to such changes, readily forming routines, such individuals are behaviourally unresponsive. Therefore, line A02h which was still characterised by a higher swimming activity pattern during Experiment 1 and lower risk taking or curiosity level during

Experiment 2 than other lines could thus be defined as low responsive line (indifferent to environmental changes). In opposite, lines A03, B45h and B61h which showed either from the start of Experiment 1 or after the stimulus fall a very strong stress response and also a high risk taking behaviour or a strong curiosity level during Experiment 2 could thus be defined as high responsive lines (very sensitive to new events or environmental changes). The cortisol level of those different lines would deserve other analyses to further investigate the relationships between behavioural and neuroendocrine stress responses in fish.

The strong negative correlations between the values for variables of the Experiment 1 and those for variables of the first test of Experiment 2 showed that these behavioural indicators are rather redundant and thus only one behavioural measurement (*i.e.* exploration in open field, flight response or initial risk taking) could be necessary to identify the personality of rainbow trout. It also revealed that it was not necessary to repeat an experiment to have a relevant evaluation of fish personality and that effort could be focused on identifying other behavioural or physiological indicators less correlated but potentially more informative.

Nevertheless, the behavioural differences observed between isogenic lines in different contexts (*i.e.* open field, flight response, risk-taking tests) clearly evidenced the existence of genetic control of a range of behavioural components in domesticated rainbow trout. Thanks to this study, isogenic lines differing for several behavioural traits were identified and constitute a relevant material for further analyses of the adaptive significance of those traits in different rearing conditions. This is a necessary step to identify the most pertinent criteria that might be used in future breeding programmes. Characterization of behavioural differences among divergent isogenic lines will facilitate also genetic mapping studies that attempt to estimate the number, position and effects of QTL responsible for these differences, as it was evidenced in zebrafish for instance (Wright et al 2006).

In conclusion, the use of isogenic lines highlighted the importance of the genetic control, in combination with life history, of the expression of personality in domesticated fish. Finally, a set of relevant trout isogenic lines with contrasted components of behavioural responses to a set of environmental stimuli has been identified and will constitute a relevant tool for further investigation on fish welfare and adaptation to farming conditions.

Acknowledgements

This research project has been supported by ANR ADD COSADD n°06-PADD-05.

The authors are grateful to Didier Leguay and Michel Prineau for their technical assistance.

References

Aragon P, Meylan S, Clobert J (2006) Dispersal status – dependent response to the social environment in the Common Lizard, *Lacerta vivipara*. Funct. Ecol. 20: 900-907

Bell A, Stamps JA (2004) Development of behavioural differences between individuals and populations of sticklebacks, *Gasterosteus aculeatus*. Animal Behaviour 68: 1339-1348

Bell AM (2005) Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). J. Evol. Biol. 18: 464-473

Berejikian BA (1995) The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator. Can. J. Fish. Aquat. Sci. 52: 2476–2482

Boissy A, Bouissou MF (1995) Assessment of individual differences in behavioural reactions of heifers exposed to various fear-eliciting situations. Appl. Anim. Behav. Sci. 46, 17-31

Boujard T, Labbe L, Auperin B (2002) Feeding behaviour, energy expenditure and growth of rainbow trout in relation to stocking density and food accessibility. Aquac Research 33: 1233-1242

Brown GE, Smith RJF (1997) Conspecific skin extracts elicit antipredator responses in juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Zool. 75: 1916-1922

Cote J, Fogarty S, Weinersmith K, Brodin T, Sih A (2010) Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). Proc. R. Soc. B: Biological Sciences 277 : 1571-1579

Dagnélie P (1975) Théorie et Méthodes Statistiques, Applications Agronomiques, Vol. 2. Gembloux: Presses Agronomiques de Gembloux.

Dingemanse NJ, Kazem AJN, Réale D, Wright J (2010) Behavioural reaction norms : animal personality meets individual plasticity. Trends Ecol. Evol. 25: 81-89

Drangsholt TMK, Damsgård B, Olesen I (2014) Quantitative genetics of behavioral responsiveness in Atlantic cod (*Gadus morhua* L.). Aquaculture. 420–421: 282-287.

Drew RE, Schwabl H, Wheeler PA, Thorgaard GH (2007). Detection of QTL influencing cortisol levels in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 272S1: S183-S194.

Feldker DEM, Datson NA, Veenema AH, Meulmeester E, De Kloet ER, Vreugdenhil E (2003) Serial analysis of gene expression predicts structural differences in hippocampus of long attack latency and short attack latency mice. Eur. J. Neurosci. 17: 379-387

Fevolden SE, Røed KH (1993) Cortisol and immune characteristics in rainbow trout (*Onchorhynchus mykiss*) selected for high or low tolerance to stress. J. Fish Biol. 43: 919-930

Fevolden SE, Refstie T, Røed KH (1991) Selection for high and low cortisol stress response in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) selected for stress response. Aquaculture 104: 19-29

Fevolden SE, Røed KH, Gjedrem B (1993) Genetic components of post-stress cortisol and lysozyme activity in Atlantic salmon; correlations to disease resistance. Fish Shell. Immunol. 4: 507-519

Giles N, Huntingford FA (1984) Predation risk and interpopulation variation in anti-predator behaviour in the three-spined stickleback, *Gasterosteus aculeatus* L. Anim. Behav. 32: 264-275

Iguchi K, Matsubara N, Hakoyama H (2001) Behavioural individuality assessed from two strains of cloned fish. Anim. Behav. 61: 351-356

Johnsson JI, Abrahams MV (1991) Interbreeding with domestic strain increases foraging under threat of predation in juvenile steelhead trout (*Oncorhynchus mykiss*): An experimental study. Can. J. Fish. Aquat. Sci. 48:243–247

Johnsson JI, Petersson E, Jönsson E, Björnsson BT, Järvi T (1996) Domestication and growth hormone alter antipredator behaviour and growth patterns in juvenile brown trout, *Salmo trutta*. Can. J. Fish. Aquat. Sci. 53:1546–1554

Koolhaas JM, Korte SM, De Boer SF, Van Der Vegt BJ, Van Reenen CG, Hopster H, De Jong IC, Ruis MAW, Blokhuis HJ (1999) Coping styles in animals: current in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23: 925-935

Krause J, Hoare D, Krause S, Hemelrijk CK, Rubenstein D (2000) Leadership in fish shoals. Fish Fish. 1: 82-89

Le Neindre P, Boivin X, Boissy A, (1996) Handling of extensively kept animals. Appl. Anim. Behav. Sci. 49: 73-81

Leblond C, Reebs SG (2006) Individual leadership and boldness in shoals of golden shiners (*Notemigonus crysoleucas*). Behaviour 143: 1263-1280

Lucas M, Drew R, Wheeler P, Verrell P, Thorgaard G (2004) Behavioral Differences Among Rainbow Trout Clonal Lines. Behav. Genet. 34: 355-365

Martin EP (1991) Individual and sex differences in the use of the push-up display by the sagebush lizard, *Sceloporus graciosus*. Anim. Behav. 41: 403-416

Millot S, Bégout ML, Chatain B (2009a) Exploration behaviour and flight response toward a stimulus in three sea bass strains (*Dicentrarchus labrax* L.). App. Anim. Behav. Sci. 119: 108-114

Millot S, Bégout ML, Chatain B (2009b) Risk-taking behaviour variation over time in sea bass *Dicentrarchus labrax*: effects of day-night alterations, fish phenotypic characteristics and selection for growth. J. Fish Biol. 75: 1733-1749

Millot S, Bégout ML, Person-Le Ruyet J, Breuil G, Di-Poï C, Fievet J, Pineau P, Roué M, Sévère A (2008) Feed demand behavior in sea bass juveniles: effects on individual specific growth rate variation and health (inter-individual and inter-group variation). Aquaculture 274: 87-95

Øverli Ø, Winberg S, Pottinger TG (2005) Behavioral and Neuroendocrine Correlates of Selection for Stress Responsiveness in Rainbow Trout—a Review. Integr. Comp. Biol. 45: 463-474

Øverli Ø, Sørensen C., Nilsson GE (2006) Behavioral indicators of stress-coping style in rainbow trout: Do males and females react differently to novelty? Physiol. Behav. 87: 506 – 512

Øverli Ø, Sorensen C, Kiessling A, Pottinger TG, Gjoen, HM (2006) Selection for improved stress tolerance in rainbow trout (*Onchorhynchus mykiss*) leads to reduced feed waste. Aquaculture 261: 776-781

Øverli Ø, Sorensen C, Pulman KGT, Pottinger TG, Korzan W, Summers CH, Nilsson GE (2007) Evolutionary background for stress-coping styles: relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. Neurosci. Biobehav. Rev. 31: 396-412

Pickering AD (1992) Rainbow trout husbandry-management of the stress response. Aquaculture 100: 125-139

Pickering AD (1993) Growth and stress in fish production. Aquaculture 111: 51-63

Pottinger TG, Carrick TR (1999) Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. Gen. Comp. Endocrinol. 116: 122-132

Pottinger TG (2006) Context dependent differences in growth of two rainbow trout (*Oncorhynchus mykiss*) lines selected for divergent stress responsiveness. Aquaculture 256: 140-147

Price EO (1998) Behavioral genetics and the process of animal domestication. In T. Grandin (Ed.), Genetics and the Behavior of Domestic Animals. San Diego: Academic Press. pp. 41–65

Price EO (1999) Behavioral development in animals undergoing domestication. Appl. Anim. Behav. Sci. 65: 245–271

Quillet E, Dorson M, Le Guillou S, Benmansour A, Boudinot P (2007) Wide range of susceptibility to rhabdovirues in homozygous clones of rainbow trout. Fish Shell. Immunol. 22: 510-519

Quillet E, Krieg F, Dechamp N, Hervet C, Bérard A, Le Roy P, Guyomard R, Prunet P, Pottinger TG. QTL for magnitude of the plasma cortisol response to confinement in rainbow trout. Animal Genetics in press.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. Biol. Rev. 82: 291-318

Rexroad C, Vallejo R, Liu S, Palti Y, Weber G (2012) QTL affecting stress response to crowding in a rainbow trout broodstock population. BMC Genetics 13: 97.

Rexroad C, Vallejo R, Liu S, Palti Y, Weber G. (2013) Quantitative Trait Loci Affecting Response to Crowding Stress in an F2 Generation of Rainbow Trout Produced Through Phenotypic Selection. Mar Biotechnol. 15: 613-27.

Riechert SE, Hedrick AV (1993) A test for correlations among fitness-linked behavioural traits in the spider *Agelenopsis aperta*. Anim. Behav. 46: 669-675

Schjolden J, Backström, T., Pulman, K.G.T., Pottinger, T.G., Winberg, S. (2005) Divergence in behavioural responses to stress in two strains of rainbow trout (*Oncorhynchus mykiss*) with contrasting stress responsiveness. Horm. Behav. 48: 537-544

Sih A, Bell A, Johnson JC (2004) Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol. Evol. 19: 372-378

Slater PJB (1981) Individual differences in animal behaviour. In: Perspectives in Ethology Vol. 4 (Ed. By D.L.G. Noakes, D.G. Linquist, G.S. Helfman & J.A. Ward). The Hague: W. Junk. pp.159-171

Sokal RR, Rohlf FJ (1995) Biometry. The Principles and Practice of Statistics in Biological Research. New York, NY: W.H. Freeman & Company.

Van Oers K, de Jong G, van Noordwijk AJ, Kempenaers B, Drent PJ (2005) Contribution of genetics to the study of animal personalities: a review of case studies; Behaviour 142: 1185-1206

Vrijenhoek RC (1994) Unisexual fish: model systems for studying ecology and evolution. Ann. Rev. Ecol. Syst. 24: 71-96

Weber GM, Silverstein JT (2007) Evaluation of a stress response for use in a selective breeding program for improved growth and disease resistance in Rainbow trout. N. Am. J. Aquacult. 69: 69-79

Weber GM, Vallejo RL, Lankford SE, Silverstein JT, Welch TJ (2008). Cortisol Response to a Crowding Stress: Heritability and Association with Disease Resistance to *Yersinia ruckeri* in Rainbow Trout. N. Am. J. Aquacult. 70: 425-433.

Wisenden BD, Chivers DP, Brown GE, Smith RJF (1995) The role of experience in risk assessment: avoidance of areas chemically labeled with fathead minnow alarm pheromone by conspecifics and heterospecifics. Ecoscience 2: 116-122

Wolf M, van Doorn GS, Weissing FJ (2008). Evolutionary emergence of responsive and unresponsive personalities. PNAS 105: 15825-15830

Wright D, Butlin R, Carlborg Ö (2006) Epistatic regulation of behavioural and morphological traits in the zebrafish (*Danio rerio*). Behav. Genet. 36: 914-922

Figure captions

Figure 1. Distance travelled (mean ± S.E., in m) measured for fish from each isogenic line and for each sequence.

Figure 2. Percentage of the total fish number per isogenic line (mean ± S.E., in %) entering in the risky zone for both tests. * indicate the isogenic lines showing significant differences.

Figure 3. (A) Total time spent (mean ± S.E., in %) by a fish in the risky zone, (B) total number (mean ± S.E.) of fish passages through the opening and (C) fish score emergence (mean ± S.E.) for both tests and for each isogenic line. * indicate the isogenic lines showing significant differences.

Figure 4. Correlations between the mean distance travelled (m) during each sequence (S1, S2, S3) of Experiment 1 and the percentage of the population entering in the risky zone (%), the time spent in the risky zone (min) and the Score Emergence during the first test (T1) of Experiment 2. The black line represents the linear regression, R the Pearson correlation value and p the statistical value.

Figure 1
Click here to download high resolution image

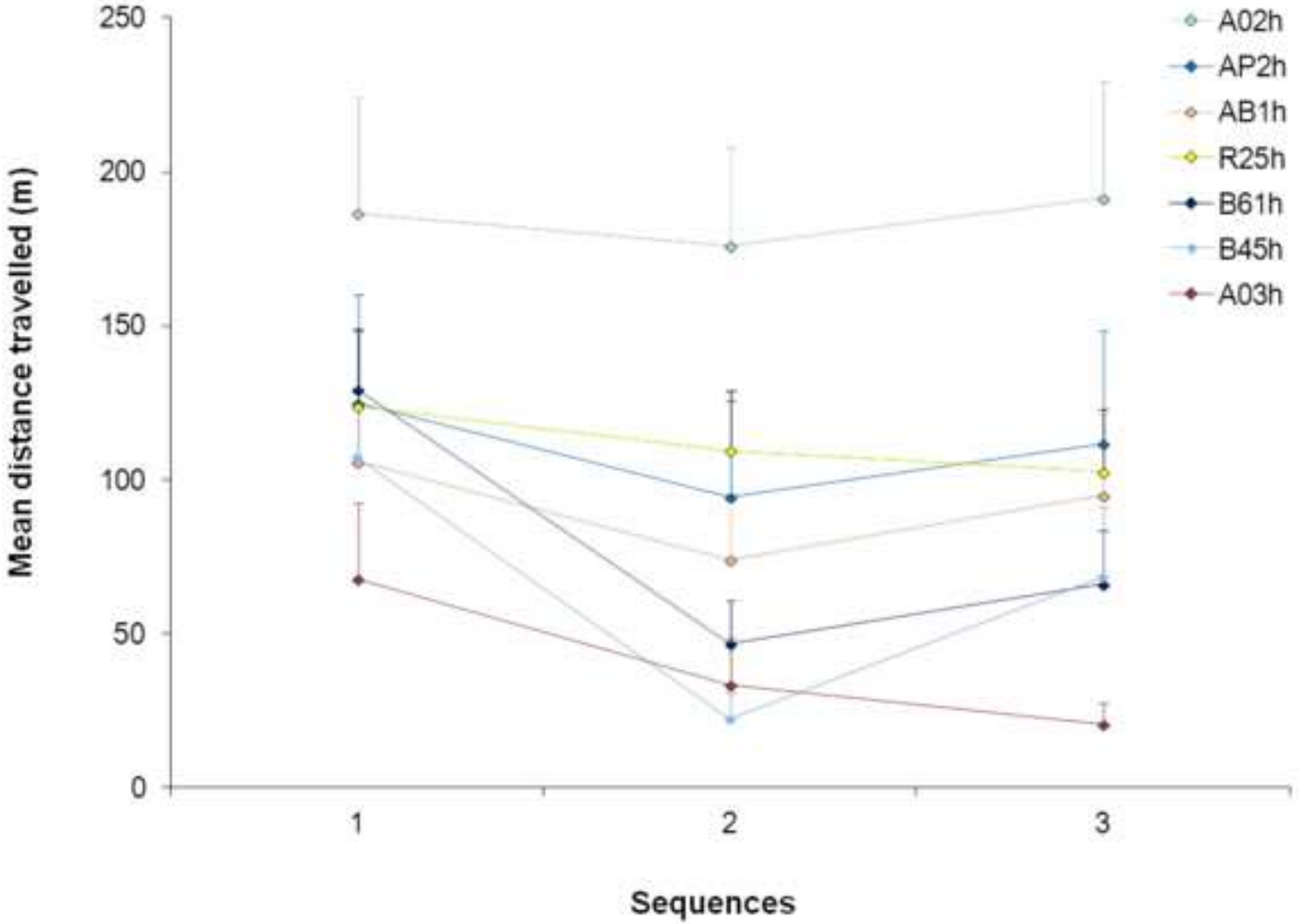

Figure 2
Click here to download high resolution image

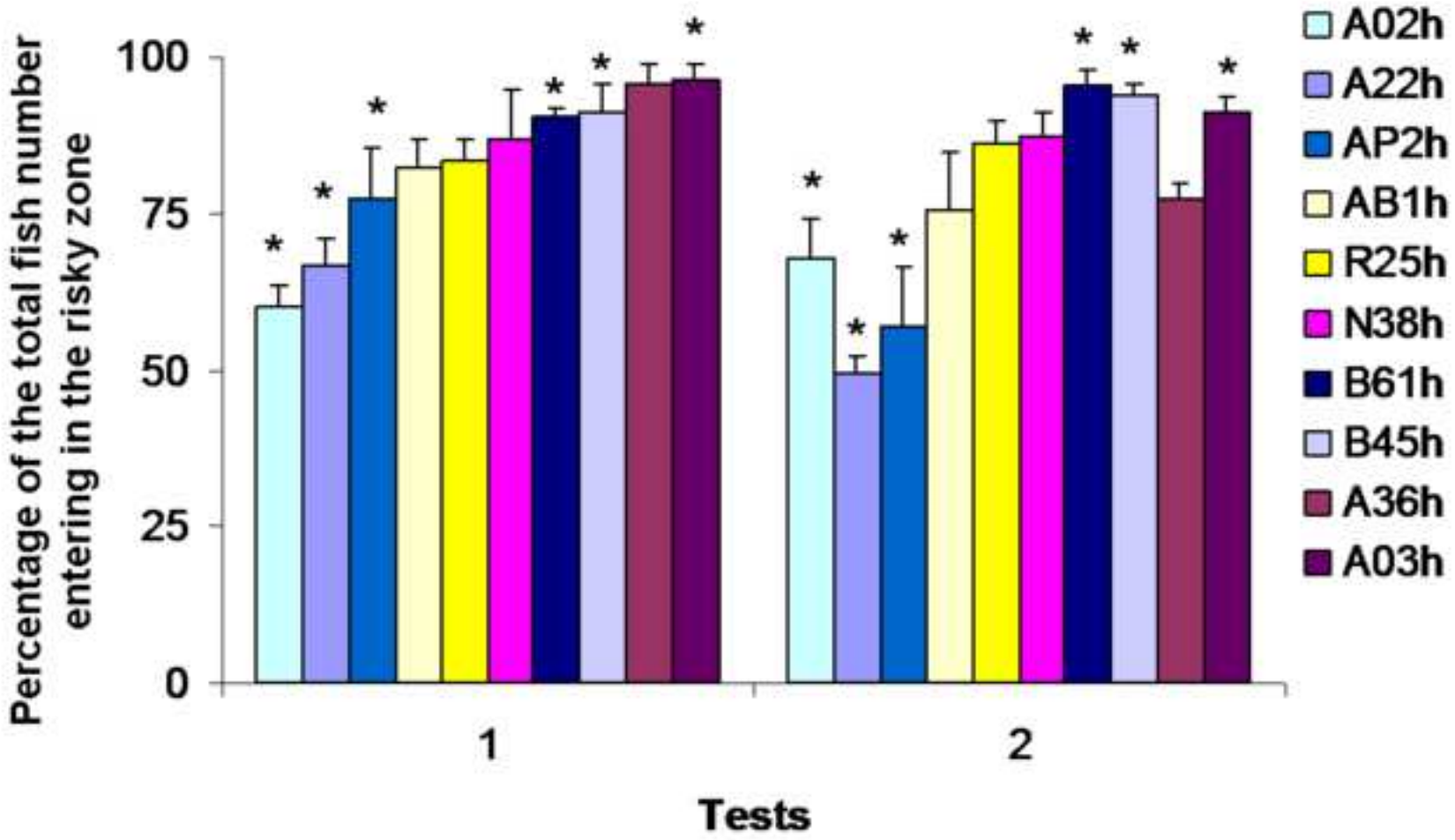

Figure 3
Click here to download high resolution image

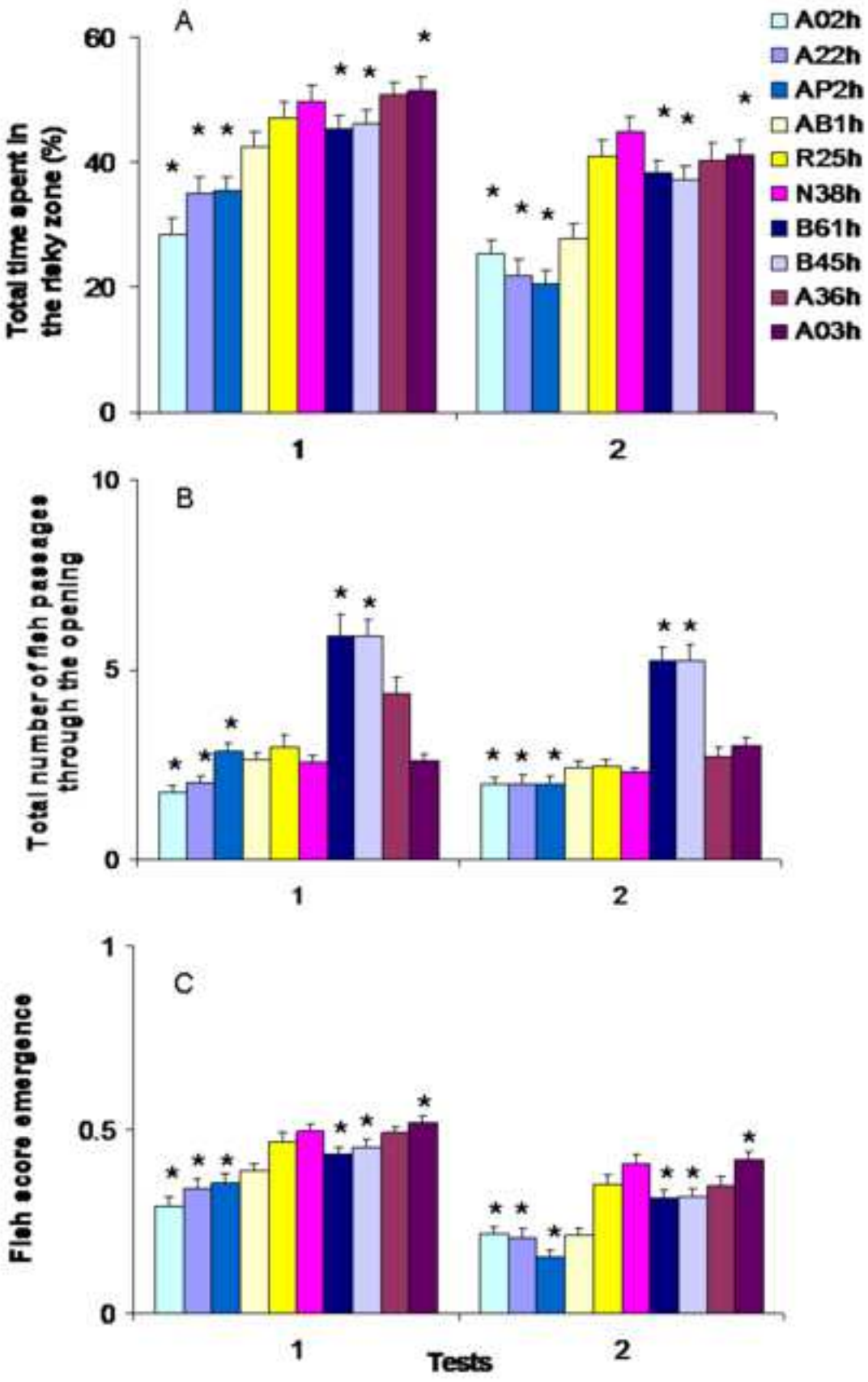

Figure 4

Click here to download high resolution image

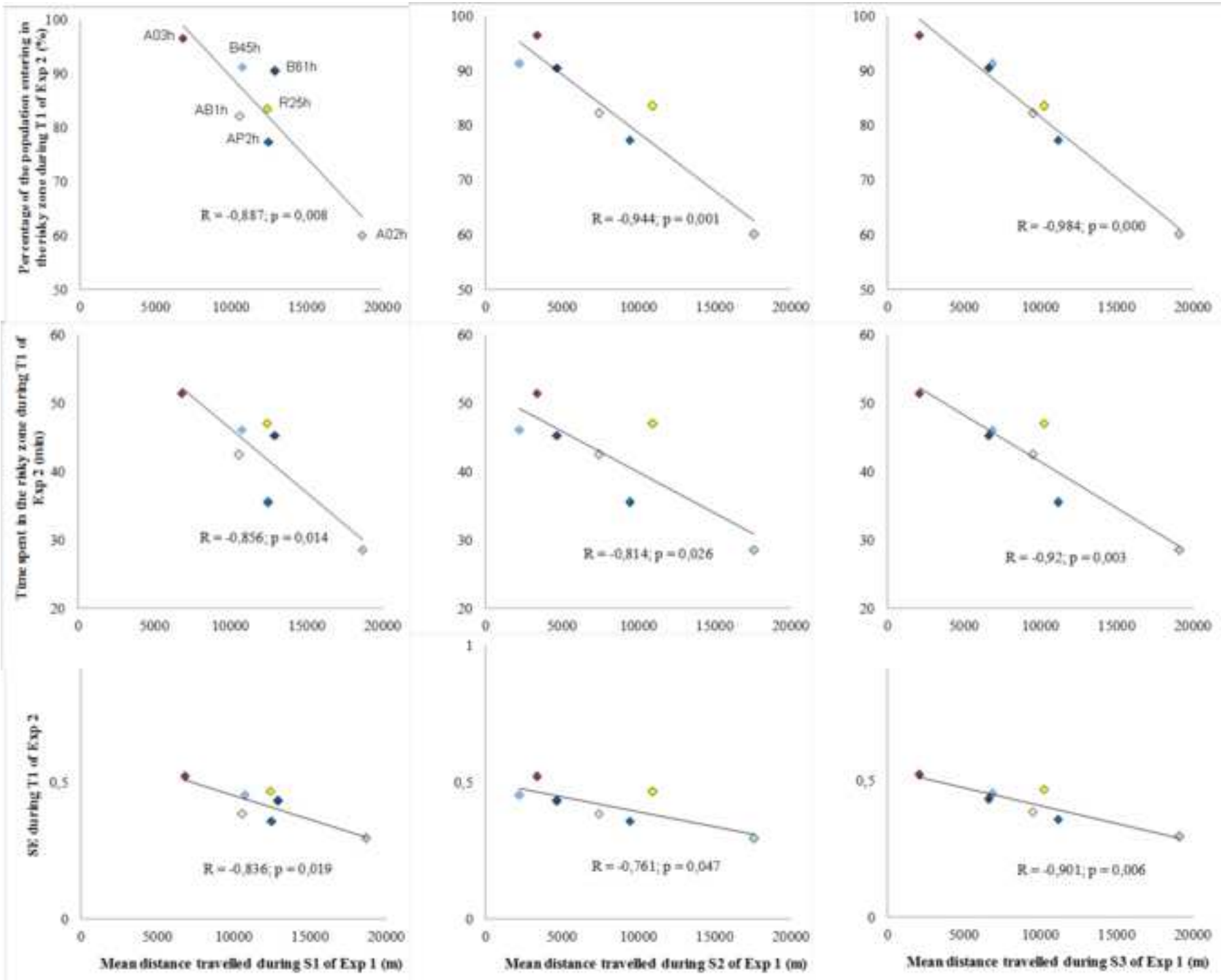

Table I. Mean (± SD) fish weight for each isogenic line and for both experiments.

	Spatial exploratory behaviour and flight response; Experiment 1		*Risk taking behaviour; Experiment 2*	
Isogenic line	Mean	SD	Mean	SD
A02h	284.2	63.1	211.6	34.3
A03h	238.3	47.7	147.2	19.3
AB1h	276.9	61.8	240.7	33.6
AP2h	262.6	43.2	236.1	31.1
B45h	207.6	37.8	170.5	23.4
B61h	223.8	23.8	176.0	19.9
R25h	281.6	29.0	222.1	35.3
A22h			192.2	26.4
A36h			169.3	25.8
N38h			232.0	33.5

Table 2
Click here to download Table: Table II.doc

Table II. Fish spatial distribution (Mean ± SE, in %) for each experimental sequence and for each isogenic line in Experiment 1.

		Sequence 1		*Sequence 2*		*Sequence 3*	
Isogenic line	Tank zone	Mean time (%)	SE	Mean time (%)	SE	Mean time (%)	SE
A02h	1	27	5	24	6	26	5
	2	39	5	41	6	43	5
	3	30	5	30	7	27	4
	4	4	1	4	1	3	1
A03h	1	11	5	2	2	2	2
	2	40	9	26	12	36	14
	3	34	9	50	13	45	13
	4	15	9	23	13	17	11
AB1h	1	20	6	14	5	17	6
	2	26	4	39	9	31	6
	3	48	8	43	10	48	9
	4	6	2	3	2	4	2
AP2h	1	14	5	17	6	15	4
	2	39	9	37	10	46	11
	3	41	9	41	12	34	10
	4	6	3	6	3	5	2
B45h	1	26	8	29	10	24	10
	2	22	4	36	10	29	8
	3	32	6	20	7	25	7
	4	20	11	15	11	22	14
B61h	1	26	8	29	9	28	8
	2	26	1	26	8	29	7
	3	43	7	38	9	40	10
	4	5	2	8	6	2	1
R25h	1	24	6	17	5	28	7
	2	40	6	38	8	31	6
	3	32	7	42	9	29	9
	4	5	2	3	1	12	9