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Unpredictability in food supply during early life influences growth and boldness in European Seabass, *Dicentrarchus labrax*

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Abstract :

Biological variability is no longer considered as statistical noise, but rather as an adaptive benefit. This variability comes from consistent differences in behavioral and physiological responses among individuals to a changing/challenging environment, named "coping style", "temperament" or "personality". Many studies have described how to characterize personality traits and how to assess their consistency over time and between different contexts; however, little is known about the environmental factors shaping personality development. Because contrasting personalities are maintained with evolution, this lead to the widespread assumption that genes play a predominant role in personality. In many cases, personality traits are however also likely to be determined by individual experience, which is probably at least as important as genetics in shaping personality. The aim of this study was to assess how environmental variability (herein food predictability) impacts behavioral responses, particularly the shyness-boldness axis, one of the most widely shared animal personality trait. Here, we reared juvenile seabass (95 days old) from two divergent strains selected for feed deprivation tolerance under standard conditions for 40 days. Thereafter, we submitted them to two feeding treatments (Predictable vs Unpredictable) starting at 135 dph and lasting 60 days. Seabass reared under a predictable food supply (PFS) grew faster and were shyer than fish reared under an unpredictable food supply (UFS) (i.e. they took more time to exit the refuge zone of a Z-maze; UFS: 132.47 ± 34.63 s; PFS: 336.79 ± 56.97 s) but their exploration tendency was similar. We also examined the behavioral responses of these fish facing a hypoxic challenge. Hypoxia tolerance results were consistent before and after the two feeding treatments. Our findings show the importance of early environmental experience as a driving force shaping boldness. In addition, we provide further evidence that predictable feeding time should be respected in studies assessing essential functions such as growth and behavior. Although personality traits are partially heritable, this study demonstrates the important influence of environmental conditions and life history on behavior

Highlights

► Early and recent experience is an important driving force shaping boldness in European seabass. ► Experiencing a predictable food supply induced faster growth and fish became shyer than fish reared under an unpredictable food supply. ► Predictable feeding time should be respected when assessing essential physiological functions such as growth and behavior.

Keywords : Personality, environmental effect, behavior, exploration, food deprivation divergent strains, *Dicentrarchus labrax*

1. Introduction

Biological variability is no longer considered as statistical noise, but rather as an adaptive benefit (Wolf et al., 2007; Castanheira et al., 2013). This variability comes from consistent individual differences mainly expressed in behavioral and physiological responses to a changing / challenging environment, named “coping style”, “temperament” or “personality” (Koolhaas et al., 1999; Sih et al., 2004; Réale et al., 2007). It covers numerous traits, such as aggressiveness, avoidance of novelty, propensity to take risks, exploration and sociability (Réale et al., 2007). One of the most studied aspects of personality is the shyness-boldness continuum (propensity to take risks) (Bell, 2007), because it occurs in a wide range of taxa (Coleman and Wilson, 1998). Bold individuals have been characterized as more aggressive/dominant (Øverli et al., 2004; Castanheira et al., 2013), faster to approach a novel object, and taking more risks than shy individuals. They explore their environment faster when exposed to novelty (Koolhaas et al., 1999; Sih et al., 2004; Korte et al., 2005; Øverli et al., 2006; Frost et al., 2007; MacKenzie et al., 2009; Réale et al., 2010), they tend to develop a behavioral routine *i.e*. show a lower magnitude of their behavioral responses with environmental changes (Bolhuis et al., 2004; Coppens et al., 2010; Ruiz-Gomez et al., 2011) and are also less attentive to their environment (Verbeek et al., 1994). By contrast, shy individuals tend to be risk averse and are generally neophobic (Verbeek et al., 1994; Wilson et al., 1994). They show higher behavioral flexibility than bold individuals (Bolhuis et al., 2004) and are more attentive facing environmental change, *i.e.* are more responsive to environmental variation (Verbeek et al., 1994). Intermediate individuals which often account for a large part of tested populations show responses that are in between these two extremes. In fish, different methods exist for measuring boldness such as the restraint test (Arends et al., 1999), the social dominance challenge (Vaz-Serrano et al., 2011), the novel object test (Frost et al., 2007; White et al., 2013) or the measure of the willingness to approach predators (Godin and Dugatkin, 1996). Exploration behavior and the latency to leave a safe area to explore a novel, less safe area (Budaev et al., 1999a; b; Fraser et al., 2001; Brown et al., 2007; Biro et al., 2010; Eriksson et al., 2010) are now also accepted as typical measures of boldness in fish and were chosen to asses boldness is our study.

Many studies have described how to characterize personality traits (reviewed in Budaev and Brown 2011; Toms et al., 2010) and others have focused on assessing the consistency of these measures over time (by repeating the same tests) and between different contexts (by combining different tests) (reviewed in Bell et al., 2009). However, few studies have investigated factors affecting personality development and shaping personality traits, as well as the influence of genetic background on personality. Indeed, genes do play a major role in personality, because the two main behavioral strategies (proactive / reactive, Koolhaas et al., 1999) persist over the course of evolution, and some behavioral and/or physiological traits associated with personality are heritable (Fevolden et al., 1993; Dingemanse et al., 2002; Fevolden et al., 2002; van Oers et al., 2004; Wright et al., 2006; Christensen et al., 2014; Millot et al., 2014). In most cases however, personality traits are probably also determined by individual experience (Sih et al., 2004), which has led some to suggest that experience is as important as genetics in shaping behavior and personality (Fox and Millam, 2004). Experiences undergone as juveniles or adults may have strong and lasting effects on personality and other behavioral traits (reviewed in Stamps and Groothuis, 2010). Experiences themselves may be related to abiotic or biotic factors. Indeed, several abiotic factors shape or influence coping style; for example, an increase in temperature of less than 3°C affects metabolic rate, resulting in higher activity and feeding rate, but also an increase in the cost of predation in Damselfishes, *Pomacentrus moluccensis* (Biro et al., 2010). Even small increases of few degrees within the space of one day can have sizeable effects on mean activity level, boldness and aggressive behavior. Stressful experiences can also affect behavioral responses. For example, Ruiz-Gomez et al., (2008) found that bold fish switch to shy fish after transportation. Hypoxia and carbon dioxide levels also affects activity and metabolism, which in turn influence behavior in European seabass (Killen et al. 2012), Rainbow trout, *Oncorhynchus mykiss* (Frost et al., 2013), Three-spined stickleback, *Gasterosteus aculeatus* (Jutfelt et al., 2013) and Coral trout, *Plectropomus leopardus* (Munday et al., 2013). Furthermore, previous studies have shown that fish from divergent stress coping style react differently to hypoxic conditions. Indeed, several studies have shown that various behavioral responses to hypoxia are associated with personality traits in Rainbow trout (Van Raaij et al., 1996; Laursen et al., 2011), Brown trout, *Salmo trutta* (Brelin et al., 2005), Gilthead seabream, *Sparus aurata* (Castanheira et al., 2013), African sharptooth catfish, *Clarias gariepinus* (McKenzie et al., 2015) and European seabass (Killen et al., 2011; Ferrari et al., 2015). Castanheira and collaborators showed that bolder Gilthead seabream escaped hypoxic conditions sooner than shyer ones and similar observations have been made for seabass (Ferrari et al., 2015). For all these reasons, we have chosen this hypoxia sorting method in our experiment along with the measures of exploration tendency and the latency to leave a safe area as proxies of boldness.

Food availability is probably another major factor that exerts long lasting effects on personality traits, because risk taking, exploration and activity level, which are three of the five main aspects of personality in animals, are related to resource acquisition (Anholt et al., 2000). Furthermore, competition for limited resources is a fundamental aspect of behavioral ecology and population dynamics (Kristensen and Closs, 2008). Fluctuation in both the quantity and quality of food during early life can have important consequences later in life (Carere et al., 2005). In this study involving a passerine bird, *Parus major*, Carere et al. found that food rationing early during life affects both exploration and aggression later in life: individuals subjected to rationing showed strong exploratory and aggressive behavior, despite four generations of selection against these traits. The predictability of food supply during early life can also affect behavior. Chapman et al., (2010) found that Trinidadian guppies, *Poecilia reticulata*, reared under conditions of unpredictable food supply spent less time in a refuge zone than fish reared with a predictable food supply and similar findings have been reported for juvenile cod, *Gadus morhua* (Braithwaite and Salvanes, 2005). Opposite results were however observed in zebrafish, *Danio rerio* (Holley et al., 2014). Here, our first objective was to assess how food predictability was affecting boldness in seabass.

Finally, genetic selection and domestication influence animal behavior (Price, 1999; 2002; Vandeputte and Prunet, 2002; Bégout Anras and Lagardère, 2004; Bouissou and Boissy, 2005; Millot et al., 2010; Benhaïm et al., 2012; Stryjek et al., 2012). Selection is mainly applied to phenotypic traits, such as growth or flesh quality, to improve production (Mignon-Grasteau and Faure, 2002; Terlouw, 2002). However, selection can also enhance or limit certain behavioral and/or physiological responses such as attack latency in mice (Veenema et al., 2005), exploration speed in birds (Stöwe et al., 2010) and cortisol responses in fish (Pottinger and Carrick, 1999). Indeed, Rainbow trout *Oncorhynchus mykiss* selected for high and low cortisol response (Pottinger and Carrick, 1999) appear to behave differently in stressful situations.

In the present study, fish came from a divergent selection based on their ability to resist fasting (*i.e.* low weight loss during starvation). It means that inter individual variability exists naturally for this trait. So the question is why do some fish have the potential to be less affected by starvation (or low food availability which is linked to food predictability in the wild)? Testing the effect of unpredictability of food supply during early life on these divergent strains could permit to better understand the ecological role of this variability. According to Damsgård and Dill (1998), food deprived animals are likely to be more willing to take risks as the costs of hiding and benefits of taking risks increase with increasing food deprivation. We could hypothesize that in environment with low food availability (or low food predictability), the potential to be fasting resistant could represent an important advantage whereas in an environment with higher food availability, this trait will be not selected for. Testing this hypothesis was our second objective. In addition, these strains were developed as indirect criteria to assess differences in feed efficiency which could be related to the same ecological relevance. A third objective was thus to evaluate the effects of genetic selection for this fasting resistance on behavior.

The aim of the present study was to assess (i) how environmental variability (herein food predictability) shape the development of personality traits, particularly boldness and exploration, ii) to test whether being tolerant to food deprivation could represent an advantage in an unpredictable food supply context and (iii) to estimate how genetic selection for food deprivation tolerance impacts behavioral responses.

2. Materials and methods

2.1 Fish and experimental conditions

Fish were hatched and reared at the experimental research station of Ifremer (Palavas-les-Flots, France) according to seabass rearing standards (Chatain, 1994). They came from a second generation of divergent strains that were selected according to their resistance to food deprivation (FD- and FD+ strains, see Dupont-Prinet et al., 2010 and Daulé et al., 2014). A sample of 600 fish (300 FD+ and 300 FD-) was transported at 86 days post hatching (dph) on 06/04/2012 to the Fish Ecophysiology Platform of La Rochelle (PEP, http://wwz.ifremer.fr/pep, France). These fish were previously fed continuously with belt feeders and commercial food. After 8 days of acclimatization (93 dph), a sub-sample of 313 fish weighing 0.86 ± 0.28 g (mean ± standard deviation (sd)) was tagged with RFID glass microtags (Ferrari et al., 2014). These fish were then distributed into six 400 L tanks located in a dedicated room, with approximately half of each strain per tank (Table 1). The six 400 L tanks were supplied with sand filtered seawater in a recirculated system (flow rate of 4 $m^3 h^{-1}$ in each tank, and 15% water renewal per day). Tanks were surrounded by an opaque black curtain to avoid any disturbance to the fish. A white light (Philips, 80W) was hung over each tank. The light cycle was controlled (14 hour day/10 hour night) throughout the experiment. Water temperature, oxygen saturation and salinity were monitored daily and maintained at optimal values 20.6 ± 0.3 °C, oxygen saturation at 75.4 ± 8.9 % and salinity at 26.9 ± 0.9 $g.l^{-1}$. Ammonia, nitrite and nitrate concentrations were checked weekly and were lower than 0.05 ± 0.05, 0.13 ± 0.06 and 0.97 ± 0.11 mg L^{-1}, respectively. From 95 dph to 134 dph (40 days), fish were hand fed between 3:00 and 5:00 PM with commercial food (61% protein and 33% lipid), according to the tables provided by the supplier (INICIOplus, BIOMAR®, France).

2.2 *Evaluation of biological performance*

Individual growth trajectories were analyzed between 93 dph and 191 dph. Biometric measurements were performed at 93, 114, 150 and 191 dph. We defined two periods of interest without disturbances to the fish (*i.e.* no manipulation): period 1 (P1), between 93 and 114 dph (*i.e.* before the treatment), and period 2 (P2), between 150 and 191 dph (*i.e.* during the treatment (predictable - PFS *versus* unpredictable food supply - UFS). The variables chosen to evaluate biological performance were body mass (BM, g) and specific growth rate: (SGR (% increase in mass per day) = 100 (Ln BM_f - Ln BM_i) / t, where BM_f and BM_i are the initial and final body mass (g), respectively, and t is the number of total days within the period).

2.3 Feeding treatments

We divided the day into four 2h time slots: from 09:00 to 11:00 AM, 11:00 AM to 01:00 PM, 01:00 to 03:00 PM, and 03:00 to 05:00 PM. Fish in the predictable treatment (PFS) were always hand-fed in the 03:00–05:00PM slot. Fish in the unpredictable treatment (UFS) were designated daily feeding slots by generating a pseudorandom sequence of numbers from 1 to 4 using the software R (with 1 representing the first slot, 2 the second slot, and so on) for each day of the feeding treatment period. Between 135 dph and 195 dph (*i.e*. 60 days), we reared fish under their assigned feeding treatments (with three tanks (T) per treatment) to investigate the importance of environmental conditions on behavior. T1, T2 and T3 were subjected to PFS whereas T4, T5 and T6 were subjected to UFS treatment. A scheme of the experimental design in presented in figure 1. At the end of the feeding treatment period, fish were hand fed each day between 09.00 to 11.00 AM.

2.4 *Behavioral trials*

2.4.1 Hypoxia test

In the hypoxia test, oxygen levels are reduced in one side of a two chambers tank and escape behavior from the hypoxic to the normoxic side is monitored (adapted from Laursen et al., 2011). The test was performed twice (hereafter called session 1 and session 2) on all the individuals.

Session 1 was performed between 121 and 124 dph, and session 2 was performed between 195 and 198 dph, just after the end of the feeding treatment (Figure 1). The hypoxia tests were carried out with two identical circular tanks (70 L, height 48 cm, diameter 49.5 cm) attached to one another via a transparent acrylic pipe (diameter 11 cm, length 30 cm, height from bottom 23 cm; see figure 1). Each tank was considered a separate environment individually equipped with oxygen and air supply that was switched off in the hypoxia tank during the trials. Fish from one home tank (T1 to T6) were gathered in one chamber of the tank (which would subsequently become the hypoxia tank) and left to acclimatize for 30 min prior to the start of the experiment. The hypoxia tank was supplied with a nitrogen source to induce hypoxic conditions during the experiment (bubbling N_2 from 90% oxygen saturation to 9 - 12% in 30 min). The second chamber of the tank under normoxic conditions (maintained at 85.1 ± 1.9 % Saturation) was referred to as the normoxic tank. A camera was placed above the apparatus to see when fish passed from one chamber to another. Oxygen level was manually recorded in the hypoxia tank every two minutes with the WTW® oxymeter. When a fish passed into the normoxic tank, it was netted, identified from its RFID tag (Nonatec© Arm reader) and the oxygen level in the hypoxia tank was noted. They were then placed in a 70 L tank before being replaced back in their respective home tank. The hypoxia test ended when 2/3 of the fish escaped from the hypoxia tank or when 10 % oxygen saturation was reached (water temperature 20°C, salinity 26.9). The variable of interest was oxygen saturation (%) in the hypoxia tank when each fish first passed into the normoxic tank. At the end of the hypoxia test, two categories of fish were defined: Fish escaping hypoxic conditions to go to normoxic tank were called Hypoxia Avoiders (HA), whereas fish staying in hypoxic tank were named Hypoxia Tolerant (HT).

2.4.2 Boldness and Exploration test

A Z-maze (70 x 45 x 15 cm) adapted from that described by Chapman et al. (2010) was used to evaluate boldness and exploration (Chapman et al., 2010). The Z-maze consisted of a refuge zone and four arms (Figure 1). Each arm was divided virtually into four quarters of equal size, referred to as quadrants. The maze thus consisted of 16 quadrants.

Fish were fed one hour prior to the test (at 09 a.m.). Then, just before the test (start at 10 a.m.), all fish from one tank were netted and placed in a 70 L tank in a dedicated room where the Z-maze was placed, allowing better efficiency of netting and no further transport. They were then randomly picked one by one from this tank and individually introduced into the refuge zone (equivalent to three quadrants, covered with a black slab and ending in a sliding door). The start area thus consisted of these three covered quadrants and the adjacent one. The fish were left for five minutes in the refuge zone and the door was opened. The fish were then allowed 15 minutes to leave the shelter. The time spent in the shelter and the latency to first exit from the shelter were taken as measures of boldness. If the individual did not go out of the shelter within this time period, the experiment was stopped and a latency of 900 s was attributed to these individuals which were not considered in the calculation of latency to leave the refuge zone. For the other fish, their activity was recorded for five minutes following the first excursion of the fish out of the refuge zone (detection in the first quadrant after the refuge zone).

Fish were recorded at a rate of 25 frames per second with an analog ICD-48E camera (Ikegami) and a 2.7–13.5 mm lens (Fujinon), linked to a PC with an acquisition card and Ethovision XT software (Noldus, The Netherlands). EthoVision XT software was also used for track extraction and analysis. The variables measured were the time to first exit the refuge zone (seconds, s) and the time spent in the refuge zone (s) during the trial. We also determined the number of quadrants into which the fish ventured within the maze (16 indicates that the fish reached the end of the maze), as an index of individual tendency to explore (Chapman et al., 2010). We converted time spent in the refuge zone and individual tendency to explore the maze into proportions which were arcsine square root transformed for statistical analysis.

The exploration tests were performed twice. Session 1 was done with 10 individuals per tank (*i.e.* 60 individuals) between 128 and 132 dph, just before the start of the feeding treatments and 7 days after the hypoxia test session 1. After the tests, fish were caught, their tags read and all individuals were returned to their respective tanks. Session 2 was initiated 3 days after the end of the feeding treatment, between 198 and 204 dph. In total 79 individuals were tested (42 UPF and 37 PFS) of which 44 were tested during both sessions (16 UPF and 28 PFS).

2.5 Data analysis

Statistical analyses were carried out with Statistica 9.0 (Statsoft, Tulsa, OK, USA) software. All data were checked for normality and homoscedasticity (Dagnélie , 1975). Before feeding treatment, body mass and SGR were analyzed using two way ANOVA with Strain as a fixed factor and Tank as a random factor. Significant differences were then analyzed by a post hoc multiple comparison test (Newman Keuls, NK). After feeding treatment, SGR were compared with two ways ANOVA using Strain as a fixed factor and Tank as a random factor nested in Treatment.

For the hypoxia test, individual oxygen levels at first passage into the normoxic tank during session 1 were compared using two way ANOVA with Strain as a fixed factor and Tank as a random factor. For session 2, individual oxygen levels at first passage into the normoxic tank were compared with two way ANOVA using Strain and Treatment (UFS, PFS) as fixed factors and Tank as a random factor nested in Treatment. Oxygen levels at first passage into the normoxic tank were also compared between the two sessions with Repeated- Measure ANOVA (RM-ANOVA) using Strain and Treatment (UFS, PFS) as fixed factors. Pearson's correlation coefficient between individual oxygen levels at first passage during sessions 1 and 2 was also calculated to assess changes at the individual level in hypoxia avoidance after the feeding treatment. A Chi ^{2} test was used to determine whether there was an effect of strain on the response to hypoxia.

In the exploration tests, fish that did not leave the refuge zone throughout the duration of the test were excluded from further analyses except for Chi^2 tests that were used to compare the proportion of fish that left or not from the refuge zone between treatments. For each variable, inter individual variability was assessed with the coefficient of variation (CV = standard deviation/mean*100, %). Inter-strain and inter-treatment differences were evaluated for time to first exit, proportion of time spent in the refuge zone and proportion of maze explored during session 1 by applying a factorial ANOVA with Strain as a fixed factor and Tank as a random factor. During session 2, we used Strain and Treatment as fixed factors and Tank as a random factor, nested in treatment. For the 44 individuals tested during both session 1 and 2, all variables were also compared between the two sessions with RM-ANOVA using Strain and Treatment (UFS, PFS) as fixed factors. Pearson's correlation coefficients were also calculated between variables of interests in the two sessions to assess changes in behavior at the individual level after the feeding treatment. Pearson correlations between order of passage and time to first exit the refuge zone were calculated to test that there was no order of passage effect on boldness variables.

Data are given as mean ± SE unless otherwise stated. A *p*-value of <0.05 was considered statistically significant.

3. Results

3.1. Growth performance

Only one fish died during the experiment, at 192 dph during the hypoxia test session 2. It came from the food deprivation intolerant strain (FD+) belonging to predictable food supply treatment group (PFS).

At the beginning of the experiment (93 dph, Table 1), there were no differences in body mass between Strain or Tank ($F_{(1,5)}=2.69$, $p=0.16$; $F_{(5,5)}=0.91$, $p=0.54$,respectively), and no interactions between factors were observed ($F_{(5,301)}=0.76$, $p=0.58$). However, SGR were different between Strain and Tank during P1 (before feeding treatment) ($F_{(1,5)}=20.13$, $p<0.01$; $F_{(5,5)}=30.61$, $p<0.001$, respectively) but no interactions between these factors were observed ($F_{(5,301)}=0.45$, $p=0.84$). Post hoc tests showed that fish from the FD+ strain had a higher SGR than fish from the food deprivation tolerant strain (FD-) during P1 (mean SGR_P1 was 3.95 ± 0.78 % for FD- and 4.19 ± 0.69% for FD+). Post hoc tests also revealed that fish in tanks 1 and 2 had significantly lower SGR (mean SGR_P1 was 3.69 ± 0.65% for T1-T2) than fish in tanks 3 to 6 (mean SGR_P1 was 4.29 ± 0.68% for T3-T6).

At the end of P2, there were no differences in body mass between Strain and Treatment ($F_{(1,306)}$=0.20, p=0.65 and $F_{(1,4)}$=0.10, p=0.77, respectively). However body mass were different between Tank ($F_{(4,306)}$=2.87, p=0.02), but post hoc Newman Keuls tests did not show any differences (p>0.05 for all tanks). SGR were different between Treatment and Tank during P2 (*i.e.* during treatment) ($F_{(1, 303)}$=4.97, p=0.026 and $F_{(4,303)}$=8.73, p<0.01, respectively) but were not different between Strain ($F_{(1, 303)}$=0.01, p=0.90). Fish from the predictable food supply treatment had a higher SGR (1.60 ± 0.004%) than fish from unpredictable food supply one (1.50 ± 0.004%) (Figure 2).

3.2. Hypoxia test

Session 1

Overall, 102 fish out of 312 (32.7%) escaped hypoxic conditions during session 1 and were called hypoxia avoiders (HA). Out of these fish 43.1% were from FD+ strain and 56.9% were from FD- strain but these proportions are not significantly different (Df=1, Chi^2=3.71, p>0.05). Oxygen saturation at first passage into the normoxic tank was 12.7 ± 1.9% for the FD+ strain and 13.1 ± 2.5% for the FD- strain (Figure 3). No significant Strain effect was observed ($F_{(1,305)}$ =3.49, p=0.06), but a Tank effect was observed on the oxygen saturation at first passage into the normoxic tank ($F_{(5,305)}$=3.45, p<0.01, data not shown). Newman Keuls post hoc tests showed that mean oxygen level when fish first passed into the normoxic tank was higher in tank 1 (13.8 ± 3.9%) than in tanks 3 and 5 (12.4 ± 1.1 %, p=0.04 and p<0.01 respectively). Tanks 2, 3, 4, 5 and 6 were not statistically different (12.7 ± 1.5 %) and Figure 3 is thus presented without data from Tank 1.

Session 2

Overall, 99 fish out of 312 (31.7%) escaped hypoxic conditions during session 2. Out of them, 46.5% were FD+ strain and 53.5% were FD- strain, these proportions are not significantly different (Df=1, Chi²=1.17, p>0.05). Oxygen saturation at first passage into the normoxic tank was 16.9 ± 13.7% for the FD+ strain and 16.9 ± 11.4 % for the FD- strain. Oxygen saturation was 13.7 ± 5.9% for UFS and 19.6 ± 15.8% for PFS fish (Figure 3). Individual oxygen saturation at first passage into the normoxic tank were not different between Strains and Treatment ($F_{(1,305)}$=0.10, *p*= 0.75 and $F_{(1,4)}$=2.64, *p*=0.20). However, a Tank effect was observed ($F_{(4,305)}$=6.73, *p*<0.001) and post hoc tests showed that tank 1 (PFS, 27.98 ± 21.20%) was different from all the other tanks (14.5 ± 6.6%). Without tank 1, mean oxygen level was 13.8 ± 5.9% for UFS and 15.6 ± 9.4% for PFS fish (Figure 3).

Session 1 versus Session 2

RM-ANOVA showed that the individual oxygen level at which fish first passed into the normoxic tank was different between Strain ($F_{(2, 186)}$=3.126, *p*=0.046), but not between Treatment ($F_{(2,186)}$=2.93, p=0.06). The oxygen level that prompted fish from the FD+ strain to move into the normoxic tank was higher during session 2 than during session 1, whereas the behavior of fish from the FD- strain remained unchanged. However, no interactions were observed between Session and Treatment for individual oxygen level ($F_{(2,186)}$=0.70, *p*=0.50). Overall, taking all fish into consideration, individual oxygen saturation at first passage into the normoxic tank was correlated between sessions 1 and 2 (70 days interval between sessions; r_p=0.22, *p*<0.001). For PFS fish, individual oxygen saturation values at first passage into the normoxic tank were correlated between sessions 1 and 2 (r_p= 0.20; *p*<0.01), but no significant correlation was observed for UFS fish ($r_{p=}$0.107; *p*= 0.20).

3.3. Exploration

Session 1

Three fish (out of 60) did not leave the refuge zone. Time to first exit the refuge zone was 68.87 ± 63.49 s; range 0-292.6 (Figure 4). The coefficient of variation (CV) was 92.19 %. There was no experimental order effect on time to first exit the refuge zone (r_p=0.01, p= 0.96). The time to first exit was not different between Strain and Tank, and there were no interactions between these factors ($F_{(1,12.7)}$= 1.14, *p*=0.31; $F_{(5,5)}$=1.19, *p*=0.43 and $F_{(5,53)}$= 0.58, *p*=0.71, respectively). Proportion of time spent in the refuge zone was 32.1 ± 32.7%; range 0.1- 95.5 % (Figure 5A). The CV was 101.7%. The proportion of time spent in the refuge zone was not different between Strain and Tank, and no interactions between these factors were observed ($F_{(1,4.5)}$=0.19, *p*=0.69; $F_{(5,5.5)}$=1.15, *p*=0.44 and $F_{(4,47)}$=0.76, *p*=0.56, respectively). The mean proportion of the maze explored was 76.8 ± 30.5% (Figure 5B); range 12.5-100%; CV=39.7%; 34 fish out of 60 reached the farthest quadrant (56.7% of fish tested explored 100% of the maze). Finally, the proportion of maze explored was not different between Strain and Tank and there were no interactions between these factors ($F_{(1,4.4)}$=0.02, *p*=0.90; $F_{(5, 3)}$=0.41, *p*=0.82 and $F_{(5,47)}$=0.87, *p*=0.49, respectively).

Session 2

Among 79 individuals tested, 21 individuals did not leave the refuge zone. Nine of these fish belonged to the UFS treatment group (42.9%) and 12 belonged to the PFS treatment group (57.1%). These proportions were not statistically different (D_f=1, Chi²=1.22, *p*>0.05).

Mean time to first exit the shelter was 132.47 ± 35.63 s; range 0-730.7 s; CV = 149.01% for UFS fish and 336.79 ± 56.97 s; range 15.8-775.2 s; CV = 84.59% for PFS fish. UFS fish emerged significantly quicker from the shelter than PFS fish ($F_{(1,51)}$ = 8.45, p<0.01, Figure 4), but no differences were observed between Strain and Tank ($F_{(1,51)}$ = 0.10, p= 0.75 and F $_{(4,51)}$ = 0.75, p=0.56 respectively). There was no experimental order effect on time to first exit the refuge zone (UFS: r=-0.01, p=0.99; PFS: r= 0.02, p=0.90). The mean proportion of time spent in the refuge zone was 41.47 ± 8.3 %; range 0.4-99.9%; CV = 95.61% for PFS fish and 41.24 ± 7.1 %; range 0.1-99.9%; CV = 97.41% for UFS fish. The proportion of time spent in the refuge zone was not different between Strain, Treatment or Tank ($F_{(1,48)}$=0.54, *p*= 0.47; $F_{(1,5.4)}$=0.64, *p*= 0.46 and $F_{(4,48)}$=0.62, *p*= 0.65 respectively, Figure 5A). 27.27% of UFS fish and 44% of PFS fish reached the farthest quadrant but the proportions were no significantly different (D_f=1, Chi²=1.76, *p*>0.05). Overall, 36.20% of fish reached the farthest quadrant in both treatment groups combined. The mean proportion of maze explored by PFS fish was 68.75 ± 8.0 % (range 6.25-100 %) with a CV of 52.15 %, whereas the mean proportion of maze explored by UFS fish was 61.91 ± 5.7 % (range 6.25-100 %) with a CV of 51.30% (Figure 5B). Neither Strain nor Treatment affected the proportion of maze explored ($F_{(1,48)}$=0.14, *p*= 0.71; $F_{(1,4.2)}$=0.02, *p*= 0.90 respectively, Figure 5B). However, a tank effect was observed ($F_{(4,48)}$=4.27, *p* < 0.01) and post hoc Newman Keuls showed that fish from tank 3 (PFS) explored significantly less of the maze than fish from tanks 1 and 2 (PFS). All other tanks were similar.

Session 1 versus Session 2

For all fish tested, time to first exit the refuge zone increased between sessions 1 and 2. Indeed, during session 1 (before treatment) time to first exit the refuge zone was 68.87 ± 63.49 s; whereas during session 2 it was 132.47 ± 35.63 s for UFS fish and 336.79 ± 56.97 for PFS fish. In addition, significantly more fish reached the farthest quadrant during session 1 (56.7 %) than during session 2 (36.2 %), (D_f=1, Chi²=4.96, *p*<0.05).

For fish tested during both sessions, time to first exit was analyzed with RM ANOVA and a Treatment effect was observed ($F_{(2,28)}$=6.99, *p*=0.01). Fish from the PFS group took longer to exit the refuge zone during session 2 than during session 1, whereas the behavior of the UFS group was unchanged. RM-ANOVA revealed that Treatment also influenced the proportion of time spent in the shelter ($F_{(2,28)}$=5.09, *p*=0.03). Indeed, fish reared under PFS treatment spent more time in the refuge zone during session 2 than during session 1, whereas the behavior of fish reared under UFS treatment was not affected. However, neither Strain nor Treatment affected the proportion of the maze explored ($F_{(2,28)}$=0.05, *p*=0.95 and $F_{(2,28)}$=1.88, *p*=0.17, respectively). Analysis of fish included in both sessions revealed no significant correlation between individual time to first exit the refuge zone during session 1 and 2 (N=32; r_p= -0.05; *p*=0.79). Similarly, the individual proportion of time spent in the refuge zone was not correlated between sessions 1 and 2 (N=32, r_p=0.16, *p*=0.39), and the same was true of the individual proportion of maze explored (N=32; r_p=-0.05; *p*= 0.79).

4. Discussion

Our results show that environmental conditions during early life influence the behavior of fish. We reared juvenile seabass (95 days old) from two divergent strains that were selected according to their tolerance to feed deprivation under standard conditions for 40 days and then submitted them to two feeding conditions starting at 135 dph and lasting 60 days. We observed that seabass reared under unpredictable food supply (UFS) during early life grew more slowly and showed bolder behavior than fish reared under predictable food supply (PFS). Overall, behavioral responses during hypoxia tests were not affected by treatment. This was also the case for exploration tendency (*i.e.* proportion of maze explored), but not for boldness (*i.e.* time to exit a refuge zone). This suggests that growth rate and boldness are rapidly affected by early environmental conditions (predictability of food supply), whereas behavioral responses to hypoxia (reflecting metabolic needs) and exploration tendency are not influenced by this particular environmental variable. Environmental variability shapes the development of personality traits, particularly boldness, faster than evolutionary processes can do, but also can modify links between behavior and physiology (herein metabolic needs).

During the feeding treatments, fish reared under a predictable food supply had a higher specific growth rate than fish reared under an unpredictable food supply. This is not surprising, because fish are able to optimize their digestive and metabolic processes by secreting digestive enzymes when meals are delivered at the same time every day (Davidson and Stephan, 1999; Stephan, 2002; Montoya et al., 2010). Thus, predictable feeding time should be respected in studies assessing growth, behavior and links between growth and other factors, such as the effect of pollutants (which often require feeding manually several batches of fish).

We also investigated the effect of selection on growth and behavior. The fish selected for food deprivation intolerance (FD+) and tolerance FD- (Daulé et al., 2014) showed differences in specific growth rate in the first period of the experiment but they rapidly disappeared during the course of the experiment. No behavioral differences were observed during the boldness and exploration tests. These results suggest that these behavioral traits are not associated with FD tolerance, although it is possible that the phenotype of FD tolerance was not sufficiently divergent between the two strains at the time of this study. Both strains thus appear to cope with the two feeding treatments in a similar manner. Indeed, the fish used in this study came from a second generation of selection and they probably still had high genetic variability, as seabass do in general. In addition, our conditions were not feed deprivation *per se*, but rather food predictability.

Hypoxia avoidance was not affected by the feeding treatment whereas this behavior is partly linked to boldness. Indeed, Killen et al. (2011) showed that risk taking behavior (*i.e.* boldness) in seabass is positively correlated with Resting Metabolic Rate (RMR) during hypoxia and Ferrari et al. (2015) showed that fish escaping hypoxic conditions (hypoxia avoider) are more active and bolder than hypoxia tolerant fish. Because the hypoxia test examines two different abilities: an individual's predisposition to taking risks (i.e. boldness) to go into the risky zone with higher oxygen concentrations, but also their ability to detect hypoxic conditions, our results suggest that physiological needs (oxygen requirements during hypoxia avoidance test) are less flexible than behavioral responses and are likely dissociable from boldness, even if boldness is linked to metabolic rate, as already demonstrated by different authors (Martins et al., 2011; Killen et al., 2011, 2012; Herrera et al., 2014). These results also accounted for that oxygen conditions (*i.e.* hypoxia) direct the risk taking response during hypoxia avoidance test (McKenzie et al., 2015). An example of flexible dissociation of behavioral and physiological component of coping style is reported in Ruiz-Gomez et al. (2008), where rainbow trout *Oncorhynchus mykiss* switched from bold to shy behavior after acute stress, although the lines used were selected for divergent behavioral and physiological responses, such that physiological responses within the population were heterogeneous and bimodal (Pottinger and Carrick, 1999). In addition, some studies have demonstrated that consistent individual differences in metabolic rate promote consistent individual differences in behavior, as reviewed in Biro and Stamps (2010). Ferrari et al. (2015) showed that seabass are particularly stressed in isolation, as they show gregarious behavior at the juvenile stage. Group-based tests are thus much more efficient than individual-based tests and consistency during hypoxia test could also be attributed to the less stressful nature of the test. In the present study, inter-strain differences only appeared during the second session, when the fish were older, with FD+ fish first passing into the normoxic tank at a higher oxygen level during the second session than during the first session, in contrast with FD- fish, which behaved similarly during both sessions. Because FD+ fish are less tolerant to feed deprivation, they may be more affected by the unpredictability of food supply, leading to an energetic trade off, herein a faster escape of low oxygen conditions (leading to an higher risk taking behavior), although the RMR of these strains are similar (McKenzie et al., 2014). This shows that being tolerant to food deprivation could be an advantage in environment with low food predictability (and availability), avoiding the needs to take more risks to get food and so increasing the exposure to potential predation risk. This results also accounting for that working on divergent selected strains could help to a better understanding of ecological consequences of selection pressures shaping behavior.

Our results about boldness in European seabass are consistent with those of Braithwaite and Salvanes (2005) on North Sea cod (another long-lived marine species): fish reared with variation in food availability took less time to start exploring a new area (time to first exit the refuge zone and start exploring the maze in our case) than fish reared with a predictable food supply. However, we did not observe any differences in exploration ability between fish reared with an unpredictable or predictable food supply, in contrast to the study of Chapman et al. (2010) on the Trinidadian guppy *Poecila reticulata*. This is probably due to an ontogenic shift in seabass behavior with age, because juveniles shoal in dense groups, move along the coast and settle progressively as they grow. Before treatment, when seabass were 128 days old, 56.7% of fish explored 100% of the maze but only 36.2%

explored the entire maze when they were 198 days old. This shows a shift in behavior due to age as also demonstrated in Ferrari et al. (2015) with another test, feeding recovery after being placed in isolation, which showed that 129 dph fish fed again whereas older fish (283 dph or 548 dph) did not. The current study provides another example of shift in behavioral response: mean time to first exit the refuge zone doubled between sessions 1 and 2, suggesting that younger juvenile seabass are bolder than older seabass, probably also in relation to their higher metabolic requirements. As reported in Abrahams and Dill, (1989), bold behavior may be advantageous when searching for new feeding areas; however, new habitats may be more risky and exploring should be avoided if an animal can acquire equivalent resources from known or less risky habitats. It is now clear that individuals are able to make behavioral tradeoffs between food intake and risk taking in a state-dependent fashion (Damsgård and Dill, 1998). When food availability is predictable, it may not pay off to take further risks such as exploring a novel environment to get food (Chapman et al., 2010). Conversely, when food availability is unpredictable, it may be more beneficial to take risks to find foraging opportunities. A hungry animal is more willing to take risks because the benefit of risk taking behavior to get food is high, which results in an increase in the willingness of the animal to pay the potential cost (risk of injury or death) (Damsgård and Dill, 1998). However, a comparison of sessions one and two, i.e. including session effects, revealed that it was not fish reared under unpredictable food supply that became bolder, but rather fish with predictable food supply that became shyer. This point was also confirmed when the results from the same individual were compared between periods. Such behavioral flexibility permits fish to adapt to their environmental conditions faster than evolution would ordinarily permit. It would be interesting to assess whether such flexibility (*i.e.* becoming bolder or shyer) is reversible and whether environmental pressure affects this reversibility.

In conclusion, we have shown the importance of early environmental quality experience as a driving force shaping boldness. In addition, we provide further evidence that predictable feeding time should be respected for studies assessing essential physiological functions such as growth and behavior. Fish experiencing a predictable food supply grew faster and became shyer than fish reared under an unpredictable food supply. Although personality traits are partially heritable, this study highlights the important influence of environmental conditions and life history on behavior.

The authors have declared that no competing interests exist.

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Figure legends

Figure 1: Scheme of the experimental design of the study. Experiment started with 93 days post hatching (dph) old fish reared under predictable food supply until the protocol changed at 135 dph after the completion of two behavioral tests. These tests were repeated again at 195 and 198 dph.

Figure 2: Specific growth rate (SGR, % of body mass gain per day) of fish during the last 45 days of the feeding treatment (P2) between 150 to 195 dph. Fish reared under predictable food supply have higher SGR than fish reared under unpredictable food supply, but no strain effect was observed (FD+, FD-: two divergent strains for feed deprivation tolerance). Values are presented as mean ± SE. Letters show significant differences at $p<0.001$.

Figure 3: Oxygen level (%, mean ± SE) at first passage into the normoxic tank during the hypoxia test before and after a feeding treatment lasting 60 days (UFS for unpredictable food supply and PFS for predictable food supply; data from Tank 1 were not included in this graph because it was different from all other tanks both Before and during the two feeding treatments).

Figure 4: Time to first exit the refuge zone (s, mean ± SE) before and after a feeding treatment lasting 60 days (UFS for unpredictable food supply and PFS for predictable food supply). Letters indicate significant differences between the two feeding treatments (PFS, UFS, $p<0.01$).

Figure 5: Exploration tests results. A- Proportion of time spent in the refuge zone (mean ± SE) and B- Proportion of maze explored (mean ± SE) before and after a feeding treatment lasting 60 days (UFS for unpredictable food supply and PFS for predictable food supply). No differences were observed between the PFS and UFS treatment group for these variables.

Figure 1:

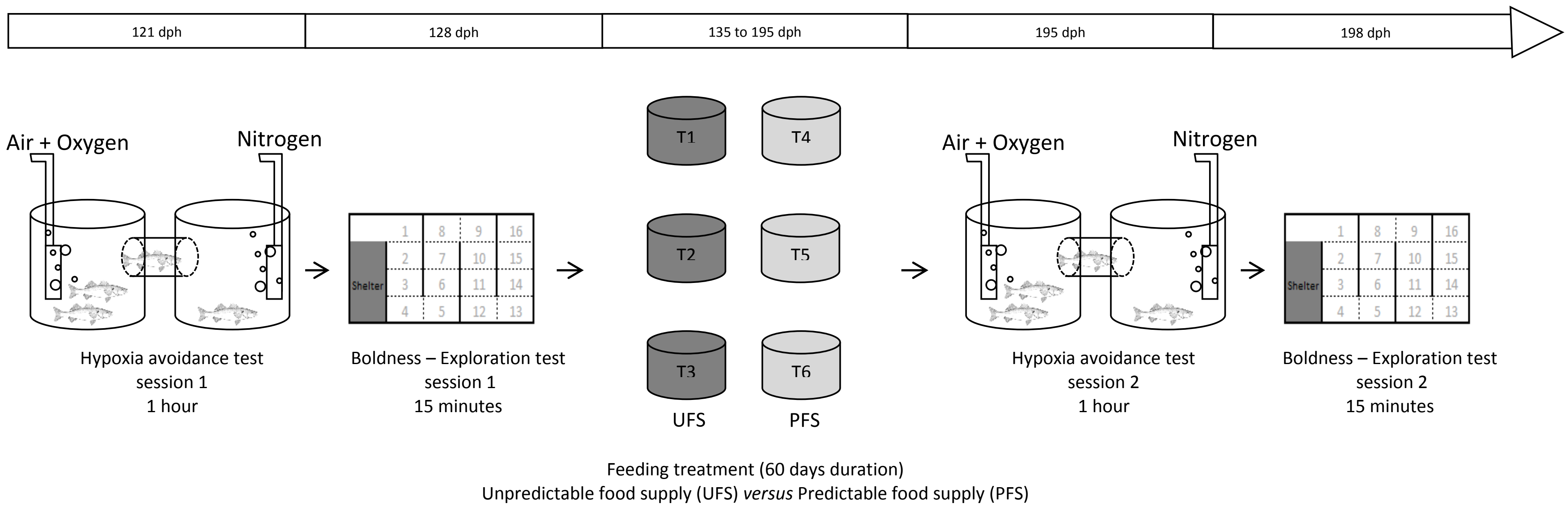
121 dph
128 dph
135 to 195 dph
195 dph
198 dph
Air + Oxygen
Nitrogen
Shelter
1 8 9 16
2 7 10 15
3 6 11 14
4 5 12 13
Hypoxia avoidance test
session 1
1 hour
Boldness – Exploration test
session 1
15 minutes
T1
T2
T3
T4
T5
T6
UFS
PFS
Air + Oxygen
Nitrogen
Hypoxia avoidance test
session 2
1 hour
Shelter
1 8 9 16
2 7 10 15
3 6 11 14
4 5 12 13
Boldness – Exploration test
session 2
15 minutes
Feeding treatment (60 days duration)
Unpredictable food supply (UFS) *versus* Predictable food supply (PFS)

Figure 2:

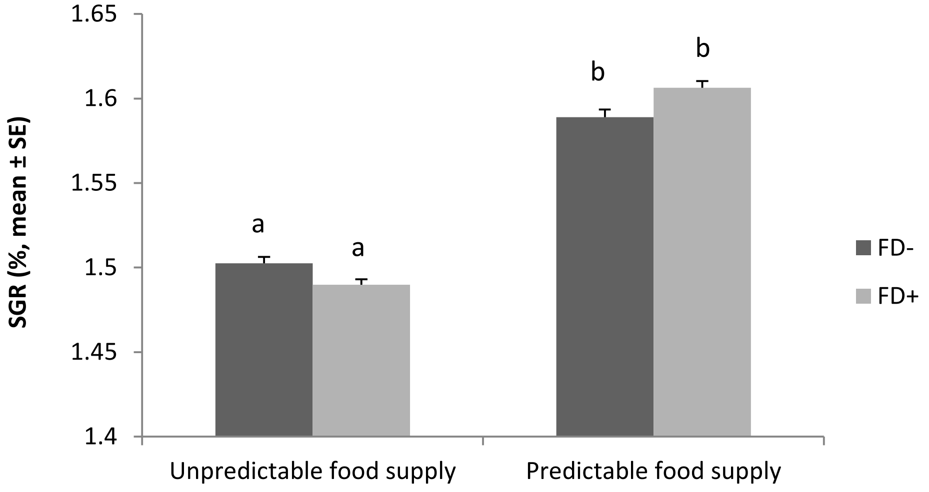
1.65
1.6
1.55
1.5
1.45
1.4
SGR (%, mean ± SE)
a
a
b
b
FD-
FD+
Unpredictable food supply
Predictable food supply

Figure 3

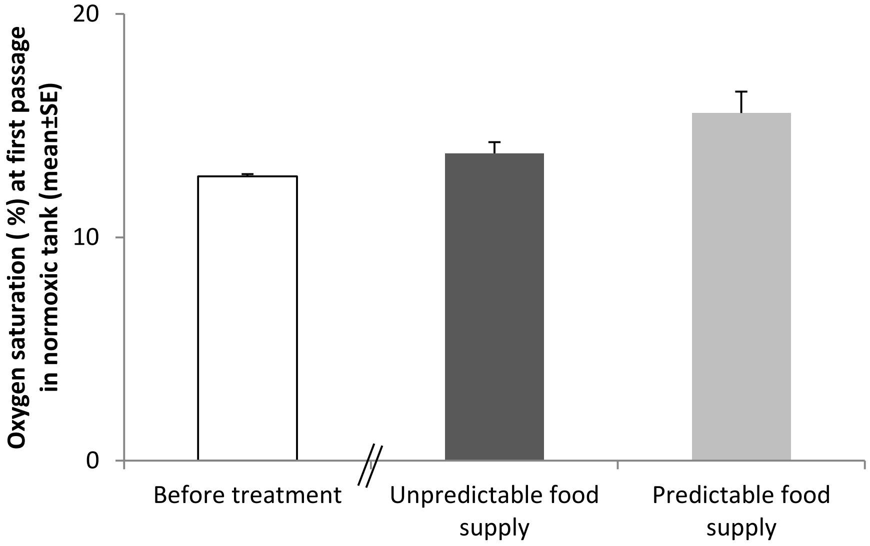
Oxygen saturation (%) at first passage in normoxic tank (mean±SE)
20
10
0
Before treatment
Unpredictable food supply
Predictable food supply

Figure 4:

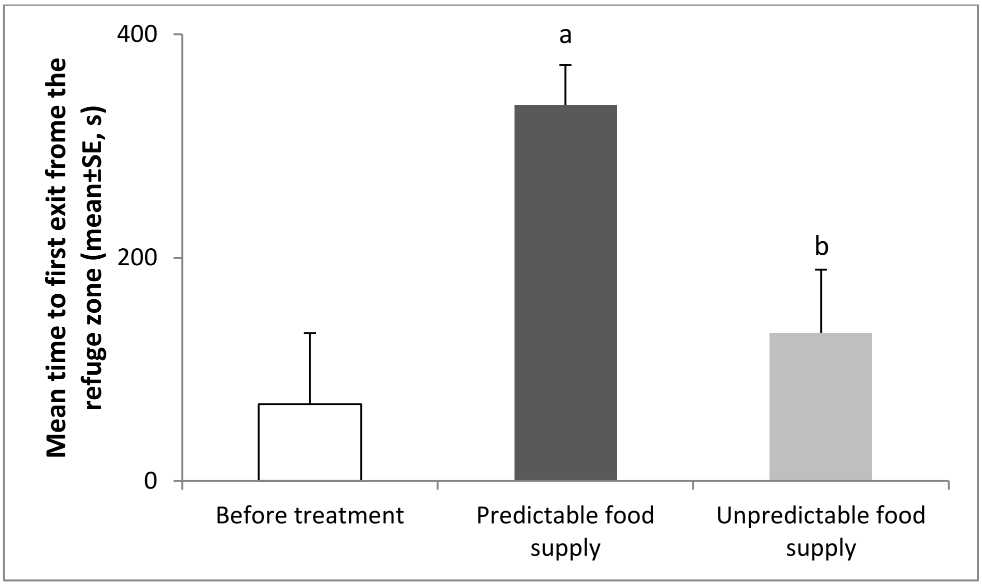
Mean time to first exit frome the refuge zone (mean±SE, s)
400
200
0
a
b
Before treatment
Predictable food supply
Unpredictable food supply

Figure 5:

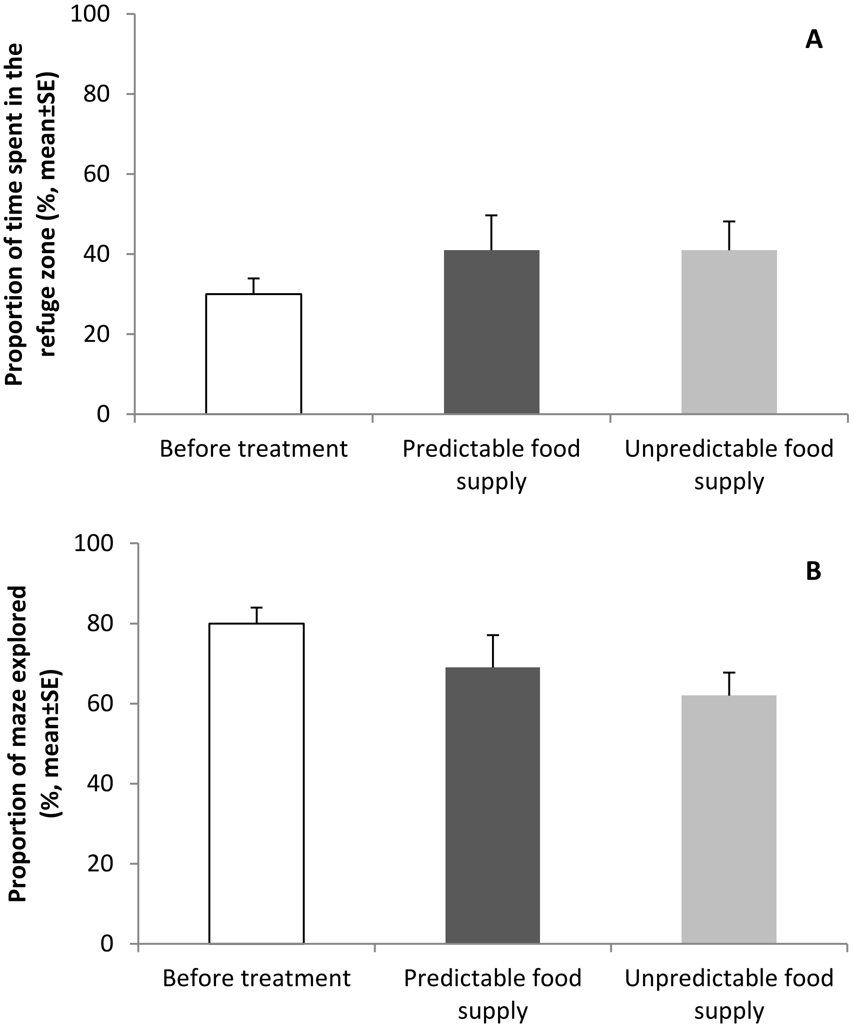
A
Proportion of time spent in the refuge zone (%, mean±SE)
100
80
60
40
20
0
Before treatment
Predictable food supply
Unpredictable food supply
B
Proportion of maze explored (%, mean±SE)
100
80
60
40
20
0
Before treatment
Predictable food supply
Unpredictable food supply

Table 1: Fish body mass (g) according to Treatment, Tank and Strain. Values are reported as mean ± sd; dph = days post hatching.

		Treatment	Predictable Food Supply						Unpredictable Food Supply					
		Tank	1		2		3		4		5		6	
	Fish age (dph)	*Strain*	FD+	FD-	FD+	FD-	FD+	FD-	FD+	FD-	FD+	FD-	FD+	FD-
Before treatment	93 dph		0.93 ± 0.19	1.00 ± 0.31	0.88 ± 0.30	0.99 ± 0.24	0.87 ± 0.19	0.91 ± 0.31	0.90 ± 0.24	1.00 ± 0.35	1.01 ± 0.28	0.96 ± 0.31	0.90 ± 0.25	0.90 ± 0.24
	114 dph		2.08 ± 0.56	2.05 ± 0.55	2.01 ± 0.71	2.10 ± 0.51	2.29 ± 0.59	2.26 ± 0.62	2.15 ± 0.43	2.49 ± 0.89	2.49 ± 0.58	2.25 ± 0.78	2.21 ± 0.67	2.11 ± 0.60
During treatment	150 dph		3.96 ± 1.29	3.67 ± 1.41	3.63 ± 1.53	4.00 ± 1.35	3.53 ± 1.35	3.95 ± 1.20	3.71 ± 0.95	4.19 ± 1.80	4.25 ± 1.42	3.76 ± 1.57	3.57 ± 1.38	3.48 ± 1.26
At the end	191 dph		8.10 ± 2.74	7.59 ± 3.12	6.87 ± 2.28	7.42 ± 2.17	6.39 ± 2.58	7.18 ± 1.97	6.34 ± 1.48	7.18 ± 2.88	8.24 ± 2.54	7.46 ± 3.46	6.77 ± 2.58	6.59 ± 2.18
		Total	30	25	32	28	27	26	23	26	26	24	26	20