Physiology & Behavior
August 2019, Volume 207, Pages 76-85
https://doi.org/10.1016/j.physbeh.2019.04.020
https://archimer.ifremer.fr/doc/00491/60282/

Coping styles in European sea bass: The link between boldness, stress response and neurogenesis

Alfonso Sébastien [1, 2, *], Sadoul Bastien [1], Gesto Manuel [3], Joassard Lucette [2], Chatain Béatrice [1], Geffroy Benjamin [1], Bégout Marie-Laure [2]

[1] Laboratoire Adaptation et Adaptabilités des Animaux et des Systèmes, IRD, UM2, CNRS, UMR MARBEC, Ifremer, Route de Maguelone, F-34250 Palavas, France
[2] Laboratoire Ressources Halieutiques, Ifremer, Place Gaby Coll, F-17137 L'Houmeau, France
[3] Technical University of Denmark, Willemoesvej 2 Building Hovedbygning, D-9850 Hirtshals, Denmark

* Corresponding author : Sébastien Alfonso, email address : Sebastien.alfonso1@gmail.com

Abstract :

Coping styles consist of a coherent set of individual physiological and behavioral differences in stress responses that are consistent across time and context. Such consistent inter-individual differences in behavior have already been shown in European sea bass (Dicentrarchus labrax), but the associated mechanisms are still poorly understood. Here, we combine physiological measurements with individual behavioral responses in order to characterize coping styles in fish. Fish were tagged and placed in a tank for group risk-taking tests (GRT) at 8 months of age to evaluate boldness using the proxy latency of leaving a sheltered area towards an open area. A subsample of these fish were individually challenged 16 months later using an open field test (OFT), in which the boldness was assessed after being placed in a shelter within an open arena. Latency to exit the shelter, time spent in the shelter, and distance travelled were recorded for this purpose. The blood and brain were then collected to evaluate plasma cortisol concentration and neurotransmitter levels (dopamine, norepinephrine, serotonin, and related metabolites), as well as brain transcription of key genes involved in stress axis regulation (gr1, gr2, mr, crf), neurogenesis (neurod1, neurod2, pcna), and neuronal development (egr1). Fish acting bolder in the GRT were not necessarily those acting bolder in the OFT, highlighting the relatively low consistency across different types of tests performed with a 16-months interval. There was, however, a significant correlation between stress markers and boldness. Indeed, mRNA levels of mr, crf, gr2, egr1, and neurod2, as well as norepinephrine levels were higher in shy than bold fish, whereas brain serotonergic activity was lower in shy fish. Overall, our study highlights the fact that boldness was not consistent over time when testing context differed (group vs. alone). This is in agreement with previous literature suggesting that social context play a key role in boldness measurement and that the particular life history of each individual may account in shaping the personality fate of a fish.

Highlights

► Boldness is not consistent when characterized using different types of test over an interval period of 16 months (group *vs.* individual tests). ► After open field test, plasma cortisol concentration is similar between bold and shy fish. ► Shy fish, however, display higher level of transcription of *mr*, *gr2*, *crf*, *egr1* and *neurod2* (trend) and also higher level of norepinephrine and lower turnover ratio between serotonin and its main metabolite (hydroxyindoleacetic acid) in the whole brain.

Keywords : Personality, HPI axis, Gene transcription, Neurotransmitters.

1. Introduction

Coping styles have been defined as "a coherent set of individual physiological and behavioral differences in stress responses consistent across time and context" by Koolhaas and colleagues [1]. They have been described in various animal species, including fish, as a *continuum* between two extreme phenotypes, called proactive and reactive [2-5]. In terms of behavior, proactive fish are bolder, more aggressive, explore their environment faster, and display less flexible behavior than reactive fish [4-8]. Moreover, correlations between these behavioral responses across time and context have been established in several fish species [2, 5, 9], as well as between behavior and physiology [4, 10-15], generally used for defining coping styles since.

Stress can be defined as the cascade of biological events that occur when an organism faces a challenge out of the normal range and attempts to reestablish physiological equilibrium [16]. Typical stress responses of fish toward adverse events involve two main neuroendocrine axes, the brain-chromaffin axis and the hypothalamo-pituitary-interrenal (HPI) axis [17-19]. Both axes are important in the primary response to stress [20-22], which is activated in various stressful contexts [21, 23-27] and leads to the release of stress hormones, catecholamines (mostly epinephrine and norepinephrine), and corticosteroids (cortisol in teleost fish) into the systemic circulation. At the central level, several monoaminergic neurotransmitters, such as serotonin (5-HT), dopamine (DA), and norepinephrine (NE), are also known to be involved in the organization of stress responses in vertebrates [27]. Stress induces changes in brain monoaminergic systems, both acutely and chronically, which is especially true for the serotonergic system. For example, serotonergic activity is consistently activated upon both chronic or acute stress exposure in fish [17, 26, 28]. Acute net chasing was for example demonstrated to induce a rapid increase in forebrain serotonergic activity in rainbow trout

[17]. Similarly, chronic confinement stress (three times a day during four weeks) also induced elevated concentration of 5HIAA and serotonergic activity in the telencephalon of Artic Charr [28]. The concentration of the brain monoamines DA, NE, and 5HT and the concentration of their associated main metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxyphenylglycol (MHPG), and hydroxyindoleacetic acid (5HIAA), respectively, are generally used to quantify monoaminergic system activity using the turnover ratio between the monoamine and associated metabolite [26, 27, 29]. This activity has been suggested to vary between individuals with different behavioral responses [26, 27, 30-32]. Both the stress axis (HPI) and monoaminergic systems (brain-chromaffin) have been shown to be reliable physiological markers correlated with differences in coping styles [7, 26, 33-35] even if, in some species, physiological markers were not directly linked to behavioral traits related to coping styles [36].

The HPI axis, which produces cortisol, its major end-product hormone, acts as an activator of physiological and behavioral responses [18, 37, 38]. Glucocorticoid receptors (Grs) and mineralocorticoid receptor (Mr) bind cortisol [39] and are detected in various fish tissues. They are involved in different physiological functions linked for instance to growth, reproduction, immunity and they participate in the regulation of control cortisol release [40-43]. The production and release of cortisol occurs in interrenal cells, located in the teleost head kidney, and is mainly controlled by adrenocortidotropic hormone (Acth), which is released by the pituitary gland into the blood [18]. In turn, the release of Acth is mainly controlled by corticotropin releasing-factor (Crf), a neuropeptide produced in the hypothalamus [20, 44]. In addition, recent studies suggest that the regulation of stress axis and neurogenesis are closely related [45-47] and we previously proposed that fish displaying high sensitivity to stress also exhibit high levels of neurogenesis in a non-stressful context [45]. Proliferating cell nuclear antigen (Pcna), neurogenic differentiation factors 1 and 2 (Neurod1

and Neurod2), and the early growth response protein (Egr1) are important markers of neurogenesis and neuronal development [34, 48]. While the regulation of stress axes might be an important feature to characterize coping styles in fish, there is still a paucity of information regarding the differences in neurogenesis between coping styles. Additional data are therefore needed in order to better understand the mechanisms associated to the behavioral responses.

Differences in behavioral and physiological responses to stress may have direct implications on growth, foraging activities, and the resulting fitness of wild fish species [37, 49, 50]. In the context of aquaculture, better knowledge of individual diversity can help to improve the welfare and management of the fish [5], as proactive fish sometimes show better feeding motivation and feed efficiency than reactive fish [2, 5, 51, 52].

The European sea bass *Dicentrarchus labrax* is a predatory species, widely distributed around Europe and West Africa [53], that has a key ecological role in the marine food chain [54]. It is also one of the most highly farmed fish species in Europe [55]. Therefore, additional knowledge of coping styles in European sea bass is required to improve both its management and welfare under conditions of aquaculture [4, 5, 56] and to better understand and predict population dynamics [57, 58].

Here, we had two main objectives. First, we monitored the boldness of the fish, by assessing the behavior of *European sea bass* over time and context testing for behavioral consistency between shy and bold fish. A group risk taking test (GRT) and open field test (OFT) were performed at 227 and 749 days *post* fertilization (dpf), respectively. Second, we aimed to describe the underlying physiological mechanisms associated with boldness. The blood and brains of the fish were sampled after the OFT to measure cortisol concentration in the plasma, the transcription of genes involved in stress regulation (*mr, gr1, gr2, crf*), neurogenesis (*pcna,*

neurod1, *neurod2*), and neuronal development (*egr1*) and, finally, the concentration of monoamines (DA, 5HT, NE) and associated metabolites (DOPAC, 5HIAA) in whole brain.

2. Material and methods

Experiments were authorized by ethics committee agreement APAFIS#7098 and all procedures involving animals were in accordance with the ethical standards of the institution and followed the recommendations of Directive 2010/63/EU.

2.1. Fish rearing and pit-tagging

The reproduction and larval rearing of European sea bass, were performed at Ifremer, Palavas-les-flots (France, 34250). Fish were produced from third-generation domesticated West Mediterranean brood stock on February 27, 2016, applying a full factorial mating design combining 10 dams and 50 sires by *in vitro* fertilization. At 223 dpf, ~3000 fish were anesthetized using 200 ppm of benzocaine (benzocaine E1501, Sigma, Saint Louis, MO, USA), measured for body mass (g) and standard length (cm), and pit-tagged in the muscle, just in front of the dorsal fin, with an ISO PIT tag (8 x 1.4 mm) to ensure individual identification.

Fish were maintained until 457 dpf following standard procedures [59]. Thereafter, they were reared in six different 5-m^3 tanks within a recirculating bio-filtered system at a temperature of 20.9 ± 1.3°C, pH = 7.4, and a salinity of 37 PSU under a 10/14 light/dark photoperiod. The fish were fed a standard marine fish aquaculture diet (Neo-grower, Le Gouessant, France) *ad libitum* using a self-feeder.

2.2. Group risk-taking test

The group risk-taking test (GRT) is a standardized test to measure boldness in fish and was adapted from Ferrari and colleagues [4]. The GRT was performed at 227 dpf with 1,468 fish

and the following day, it was repeated with the other 1,468 naive fish (mass = 18.8 ± 1.2 g; standard length = 10.3 ± 1 cm).

The GRT consists of placing all the fish into one half of a 1.5 -m^3 tank, separated in two by an opaque PVC divider (**Figure 1.A**). Fish were gently transferred from their home tank to one half of the experimental tank under light anesthesia (100 ppm Benzocaine, same stock solution as before) between 10:00 and 11:00 am. The side containing the fish was covered with a black opaque curtain to create a sheltered area. Both halves of the tank received water of the same quality, oxygenation, and exchange rate. After an habituation period of 2 h, the door (a circular opening of 12 cm Ø in the centre or the divider) was opened and the fish were left with the possibility to exit the sheltered area, therefore exhibiting a risk-prone behavior reflecting boldness level. The door was equipped with a PIT-tag detection antenna (DORSET, The Netherlands) connected to a control device. The PIT-tag detection antenna allowed the monitoring of fish behavior during the GRT by obtaining the exact exit time (*latency to exit (s)*) of each fish leaving the sheltered area for the first time. As the test lasted for 24 hours, a latency of 1,440 min was assigned to fish that did not exit the sheltered area before the end of the test. Once the outcome of the GRT was analyzed, the individuals were categorized as bold (fish that exited the shelter) or shy (fish that stayed in the shelter during the entire experiment). They were then transferred back to their home tanks and reared together until the open field test (OFT) was performed, 16 months later.

2.3. Open field test

The open field test (OFT) was adapted from that of Benhaim and colleagues [60] and performed at 749 dpf (341 ± 4 g) using a sub-sample of fish (n = 57, half of them bold and the other half shy, based on their GRT categorization). The OFT consists of placing an individual fish in an open and novel arena (75 x 75 cm with a water height of 23 cm, **Figure 1.B**) and tracking its swimming activity. The fish was placed in the closed sheltered area for a

habituation period of 5 min. The door was then opened, allowing the fish to explore its novel environment while being video-tracked. Videos were taken with a DMK 31AU03 camera (The Imaging Source, Germany) and the recorded behavior of each fish then analyzed using EthoVision XT 13.1 software (Noldus, The Netherlands) by the same operator (SA). The *latency to exit* the shelter (s) was recorded for each individual. A latency of 1,200 s was assigned to fish that did not exit the shelter at the end of the test. Fish that exited the shelter were categorized as bold, whereas those that did not were categorized as shy. The *total distance travelled* (cm), *number of transitions* between shelter and arena, and the *time spent in the shelter* (s) were also recorded.

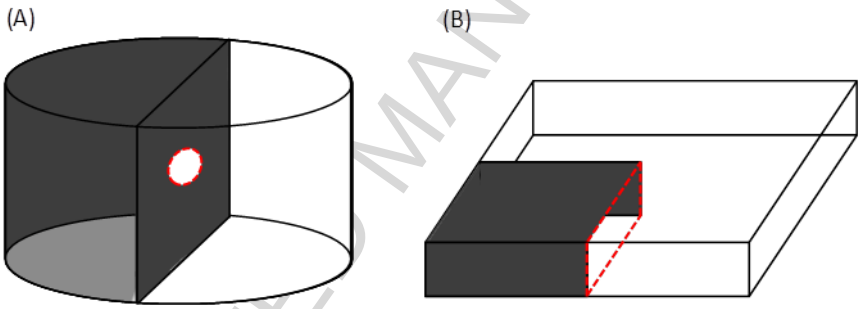

Figure 1. Design of the apparatus for the (A) group risk-taking test (GRT) and (B) open field test (OFT). Dark grey boxes represent the shelter; dashed red lines indicate the door position.

2.4. Blood and brain sampling

Following the OFT, fish were gently removed from the arena and euthanized using 1,500 ppm benzocaine. Blood and brain were sampled without knowing the boldness level of the fish. Blood was sampled from the caudal vessels using lithium heparinized syringes [61]. Then, blood was centrifuged (4 min at 13,000 x g) to obtain plasma samples, which were stored at -20°C until analysis. The plasma cortisol concentration was determined by ELISA (RE52061,

IBL International, Hamburg, Germany) using a Synergy-HT microplate reader (BioTek Instruments, Winooski, VT, USA) following manufacturer's instructions.

The brain was extracted, divided along the sagittal plane, and placed directly in liquid nitrogen. The left half was used for monoamine analyses and the right half for gene transcription analyses.

2.5. Brain monoamine analyses

The left half of the fish brain was used for HPLC analysis of brain monoamines. Tissues were weighed and immediately homogenized in 1 mL perchloric acid (PCA) solution (0.4 M PCA and 0.1 mM EDTA) with a Sonopuls ultrasonic homogenizer (Bandelin, Germany). Homogenates were then centrifuged for 10 min at 14 000 x g (at 4°C). Supernatants were diluted 20 times in the PCA solution and 20 µL of the dilution injected into the high performance liquid chromatography (HPLC) system.

The diluted supernatants were analyzed high performance liquid chromatography with electrochemical detection (HPLC-EC). The HPLC mobile phase consisted of 73.9 mM NaH_2PO_4, 0.1 mM Na_2EDTA, and 0.58 mM sodium 1-octanesulfonate in deionized water with 15.3% (v/v) methanol. The pH of the mobile phase was adjusted to 3.0 with orthophosphoric acid. The chromatographic separation was performed at 0.9 mL/min with a Reprosil-Pur 120 C18-AQ column (150 x 4 mm, 5 µm particles) (Dr. Maisch, Germany) and electrochemical detection of the separated compounds was performed using an ESA Coulochem II detector. The detection system included a double analytical cell with oxidation potentials set at 0 mV (first electrode) and +340 mV (second electrode). A conditioning cell at + 400 mV was used before the analytical cell to oxidize potential interfering compounds. The rest of the HPLC system consisted of a Shimadzu LC-10AD pump and a Midas autosampler

(Spark Holland, The Netherlands). Acquisition and integration of the chromatograms were performed using ClarityTM HPLC software (DataApex Ltd, Czech Republic).

The levels of serotonin (5HT), dopamine (DA), and their main oxidative metabolites 5-hydroxyindoleacetic acid (5HIAA) and 3, 4- dihydroxyphenylacetic acid (DOPAC) were quantified, along with the levels of norepinephrine (NE). The main NE metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), was below the detection limit. Quantification was carried out by comparing peak areas with those of the corresponding standards. The ratios between 5HIAA and 5HT and between DOPAC and DA were then calculated as indirect measures of the activity of the serotonergic and dopaminergic neurons, respectively [27].

2.6. Gene transcription analyses

The right half of the fish brain was used for gene transcription analyses.

Extraction and reverse transcription of total RNA from the brain

Each half-brain (± 200 mg) was mechanically disrupted using a ball mill in 900 µL QIAzol lysis Reagent (Quiagen) in 2 ml RNAse-free tubes. They were then diluted two-fold before starting RNA extraction, following the manufacturer's instructions (RNeasy Plus Universal Mini Kit protocol, Qiagen, recommended initial sample size: 100 mg). The reverse transcription of total RNA to cDNA was performed as described in Geffroy and colleagues [62]. RNA quantity was assessed by measuring the A260/A280 ratio using the NanoDrop® ND-1000 V3300 Spectrophotometer (Nanodrop Technology Inc., Wilmington, DE, USA). Briefly, an aliquot of each tube was diluted in diethylpyrocarbonate-treated water. Then, 1.5 µg RNA was reverse transcribed using 200 U Moloney murine leukemia virus (MMLV) reverse transcriptase (PROMEGA) and 2 µg random hexamers (Promega) in a master-mix buffer and supplemented with 25 U RNase inhibitor (RNasin; Promega) in a final volume of

15 µl. All cDNA were diluted 50 fold in nuclease-free water prior to quantitative real-time PCR (qPCR).

Quantitative real-time PCR

The transcription of genes involved in stress regulation, namely glucocorticoid receptor 1 and 2 (*gr1* and *gr2*), the mineralocorticoid receptor (*mr*), and corticotrophin releasing factor (*crf*) was monitored. We also investigated the expression of genes involved in neurogenesis: neurogenic differentiation factors (*neurod1* and *neurod2*) and proliferating cell nuclear antigen (*pcna*), as well as an early growth response gene (*egr1*), involved in brain development.

Primer sequences were retrieved from the literature [45, 63, 64] or specifically-designed using the web version of PRIMER 3 (http://primer3.ut.ee; **Table 1**). The qPCR experiments were conducted in a final volume of 1.5 µl, using 1 µl fish brain cDNA and 0.5 µl of an optimized concentration of primers and Fast SYBR Green PCR Master Mix (Applied Biosystems, Life technologies). Distribution of the volume into the plate was performed using a robot (Labcyte Echo525) able to distribute as low as 25 nL by acoustic transfer ensuring reliable repeatability even with small qPCR volumes. The hot start enzyme was activated for 2 min at 95°C and amplification carried out using the following program: 95°C for 3 s and 60°C for 30 s for 40 cycles. After amplification, a melting curve was obtained using the following protocol: 10 s holding at 55°C followed by sequential 0.05°C increases repeated 80 times. Technical duplicates were run for each sample. A standard curve was generated by mixing all samples and the efficiency (E) for all genes was calculated (*ef1*: 1.98; *l13*: 1.96; *gr1*: 1.90; *gr2*: 2.14; *mr*: 2; *crf*: 1.83; *egr1*: 2; *neurod1*: 1.88; *neurod2*: 1.88; and *pcna*: 1.80).

Housekeeping genes

Ribosomal protein (*l13*) and eukaryotic translation elongation factor 1 alpha (*eef1-alpha*) have both been recommended as housekeeping genes [65, 66] and were used in this study. The coefficients of variation were below 5% for both *l13* and *eef1-alpha*. The amplification efficiency was 98 and 96 % for *l13* and *eef1-alpha* respectively.

Relative levels of gene transcription were obtained using the following equation with the efficiency (E) of the target gene normalized by the efficiency and the geometric mean of the two housekeeping genes as reference (ref; *l13*and *eef1-alpha*), according to Roche diagnostic [67]:

$$Relative\ level\ of\ gene\ transcription = \frac{(E_{ref})^{Ct_{ref}}}{(E_{target})^{Ct_{sample}}}$$

Table 1. GenBank accession numbers and primer sequences for each gene used for gene transcription analyses.

Gene	GenBank accession numbers	Forward primer (5' → 3')	Reverse primer (3' → 5')
mr	JF824641.1	GTTCCACAAAGAGCCCCAAG	AGGAGGACTGGTGGTTGATG
gr1	AY549305[a]	GAGATTTGGCAAGACCTTGACC	ACCACACCAGGCGTACTGA
gr2	AY619996[a]	GACGCAGACCTCCACTACATTC	GCCGTTCATACTCTCAACCAC
crf	JF274994	GCAACGGGGACTCTAACTCT	GTCAGGTCCAGGGATATCGG
neurod1	b*	TTCTCCTTCAGCGTGCACTA	GGTGCGAGTGTCCATCAAAG
neurod2	d*	TGCGTAAAGTGGTTCCATGC	GTCGTGGGTTGGGAGAGTC
pcna	JQ755266[c]	CAGAGCGGCTGGTTGCA	CACCAAAGTGGAGCGAACAA
egr1	d*	AACTCCAGCCTCAGTTCCTC	AGTCAGGAATCATGGGCACA
eef1-alpha	AJ866727.1	AGATGGGCTTGTTCAAGGGA	TACAGTTCCAATACCGCCGA
l13	DT044910.1	TCTGGAGGACTGTCAGGGGCATGC	AGACGCACAATCTTGAGAGCAG

a*: from Pavlidis and colleagues [63]; b*: from Sadoul and colleagues [45]; c*: from Crespo and colleagues [64]; and d*: designed from the European sea bass genome from UCSC.

2.7. Statistical analyses

Statistical analyses were performed using R 3.1.0 software [68]. All statistical analyses were carried out at the 95% level of significance. Values are expressed in the text as the mean ± standard error of the mean (SEM) and illustrated in the figures using boxplots. The central line of the boxplot indicates the median and the boxes the quartiles, with the whiskers covering 95% of the values. Outliers are represented by points. First, the chi-square test was performed to compare the proportion of bold versus shy fish obtained between the GRT and OFT. Then, an exact binomial test was performed to evaluate behavioral consistency between

the GRT and OFT by comparing the proportion of consistent behavior with random theoretical probability ($p = 0.5$) for each coping style. The remaining tests (concerning physiology and behavior) were performed on fish screened in the GRT and the OFT respectively.

Normality was verified for other comparisons between coping styles on behavioral and physiological variables using the Shapiro test before applying either the Student's t-test or Wilcoxon rank sum test, depending on whether normality was verified or not. Comparisons between individual coping styles in the *distance travelled*, the *number of transitions* between shelter and OFT arena, and the *time spent in the shelter* were performed using the Wilcoxon rank sum test.

Concerning physiological variables, two individuals were discarded due to an RNA extraction failure (no gene transcription) and therefore analyses were performed on a total of 55 individuals. The Student's t-test was performed to compare plasma cortisol concentrations and monoamine concentrations in the brain (5HT, 5HIAA, 5HIAA/5HT turnover ratio, DA, DOPAC, DOPAC/DA, and NE) between coping styles measured in the OFT. The Wilcoxon rank sum test was performed to compare the transcription of genes (*gr1*, gr2, *mr*, *crf*, *neurod1*, *neurod2* and *pcna*) between coping styles (Student t-test for *egr-1*).

Finally, a principal component analysis (PCA) was performed among fish that exited the shelter with individual continuous variables recorded in OFT (Latency to exit shelter, distance travelled, time spent in shelter and number of transitions) using ade4 package [69]. Individual PC scores of the two first axes of the PCA were then downloaded. PC scores from axis 2, reflecting coping styles were analyzed using linear regression as in relation to either monoamine concentration or relative level of gene transcription in the brain. Homogeneity of the model residuals was *a posteriori* checked using Shapiro-Wilk.

3. Results

3.1. Behavioral consistency across context and time between the GRT and OFT

During the GRT, 63% of the fish exited the sheltered area within the 24 hours of the test and were designated as bold. Consequently, 37% of the fish were designated as shy in the GRT. In the OFT, 58% of the fish exhibited bold behavior and exited the sheltered compartment *vs.* 42% which exhibited shy behavior and stayed in the sheltered compartment during the 20 min of the test. The proportion of fish with bold/shy behavior was similar between the GRT and OFT (χ^2 = 0.51; p = 0.48, **Figure 2A**). However, there was no behavioral consistency across context between the GRT and OFT for either bold or shy fish designated as such during the GRT (p = 0.28 and 0.85, respectively). Indeed, 46% of fish designated as shy during the GRT stayed shy in the OFT, whereas the other 54% became bold one year later in the OFT. For fish designated as bold during the GRT, 61% stayed bold, whereas the other 39% became shy during the OFT (**Figure 2B**).

Table 2. Contingency table showing behavior displayed by the fish during the two tests (GRT and OFT).

		Open field test		
		New shy	New bold	Total
Group risk-taking test	Original shy	12	14	26
	Original bold	12	19	31
	Total	24	33	57

3.2.Open field test

Of the 57 fish screened in the OFT, 58% were categorized as bold. These bold fish actively swam in the tank during the 20 minutes of the test (912.7 ± 60.2 cm; W = 792, p < 0.001) and moved between shelter and open field area (2.4 ± 0.4 times; W = 0, p < 0.001); whereas the

shy fish stayed in the shelter. Although the bold fish exited the shelter and were active in the open arena, most nonetheless spent most of the test time in the shelter (1,000 ± 64 s), but still for less than the shy fish (W = 792, p < 0.001, **Figure 2**).

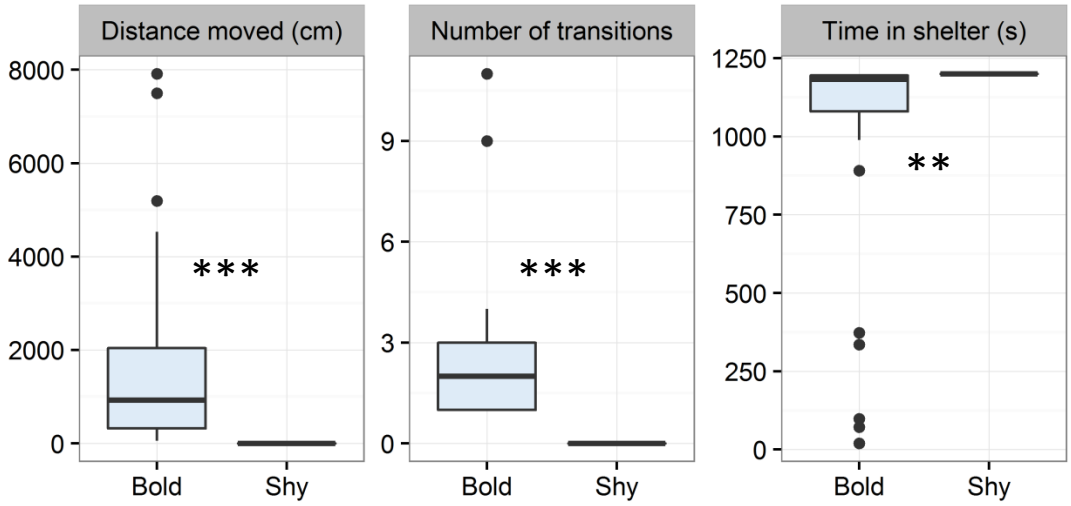

Figure 2. Distance travelled (cm), number of transitions between shelter and arena, and time spent in the shelter (s) during the open field test (OFT) between bold and shy fish categories as assigned in the OFT. Wilcoxon rank sum test: **: p < 0.01; ***: p < 0.001.

3.3. Plasma cortisol concentration

Plasma cortisol concentrations were similar between bold and shy fish screened in the OFT (t = -0.10, p = 0.92, data not shown). The mean plasma cortisol concentration in bold fish was 904.3 ± 51.8 ng/mL (n = 33) and 912.7 ± 60.2 ng/mL in shy fish (n = 24).

3.4. Monoamine concentration in the brain

There was no difference in the concentration of DA or DOPAC in the brain between bold and shy fish screened in the OFT, nor the DOPAC/DA ratio (t = -0.86, p = 0.40; t = -0.58, p = 0.57; and t = -0.61, p = 0.54, respectively). For the serotonergic system, there was no difference in the concentration of 5HT or 5HIAA in the brain between bold and shy fish (t = 0.45, p=0.66 and t = -1.15, p = 0.25, respectively). However, shy fish had a significantly

lower 5HIAA/5HT ratio in the brain than bold fish (t = -2.17, p = 0.03). The concentration of NE in the brain was also significantly higher in the shy than bold fish (t = 2.22, p = 0.03, **Figure 3**). Note that when considering bold and shy fish categories as assigned after the GRT, no difference in monoamine concentration or turnover ratio was detected (**Supplementary material**).

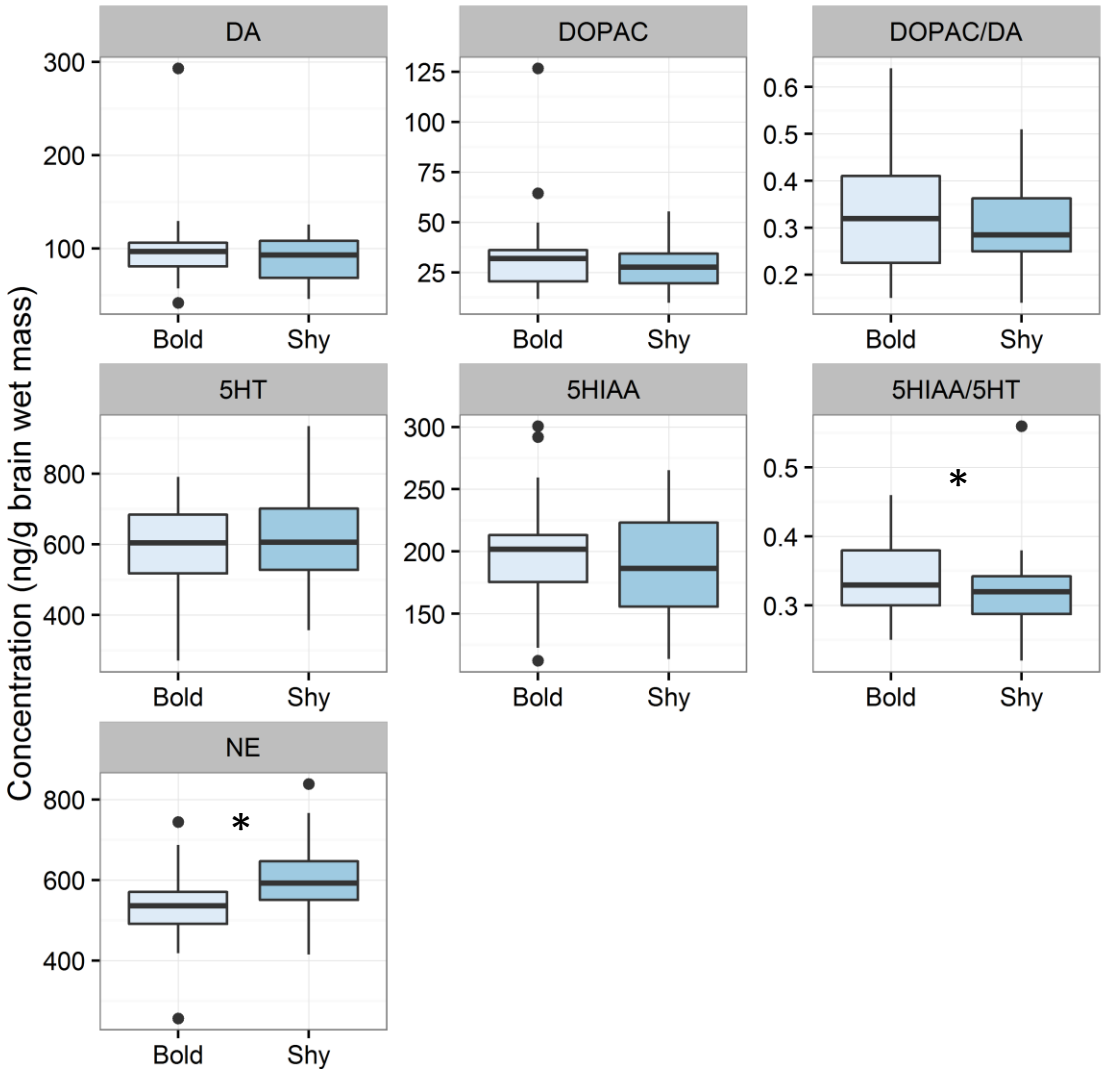

Figure 3. Concentration (ng/g brain wet mass) of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), the DOPAC/DA turnover ratio, serotonin (5HT), 5-hydroxyindoleacetic acid (5HIAA), the 5HIAA/5HT turnover ratio, and norepinephrine (NE) in the brains of bold and shy fish categories as assigned in the open field test (OFT). Student's t-test: *: $p < 0.05$.

3.5. Gene transcription in the brain

The transcription of *gr1* in the brain did not differ between bold and shy fish screened in the OFT ($W = 312$, $p = 0.32$), whereas *gr2*, *mr* and *crf* were significantly upregulated in the brains of shy fish relative to those of bold fish ($W = 171$, $p = 0.01$, $W = 255$, $p = 0.047$ and $W = 145$, $p < 0.001$, respectively).

The transcription of *neurod1* and *pcna* in the brain did not differ between bold and shy fish ($W = 358$, $p = 0.82$ and $W = 311$, $p = 0.31$, respectively), whereas there was a trend towards the upregulation of *neurod2* in shy fish relative to bold fish ($W = 269$, $p = 0.08$). The transcription of *egr1* was significantly upregulated in the brains of shy fish relative to those of bold fish ($t = -2.70$, $p < 0.01$, **Figure 4**). Note that when considering bold and shy fish categories as assigned after the GRT, no difference in gene transcription was detected (**Supplementary material**).

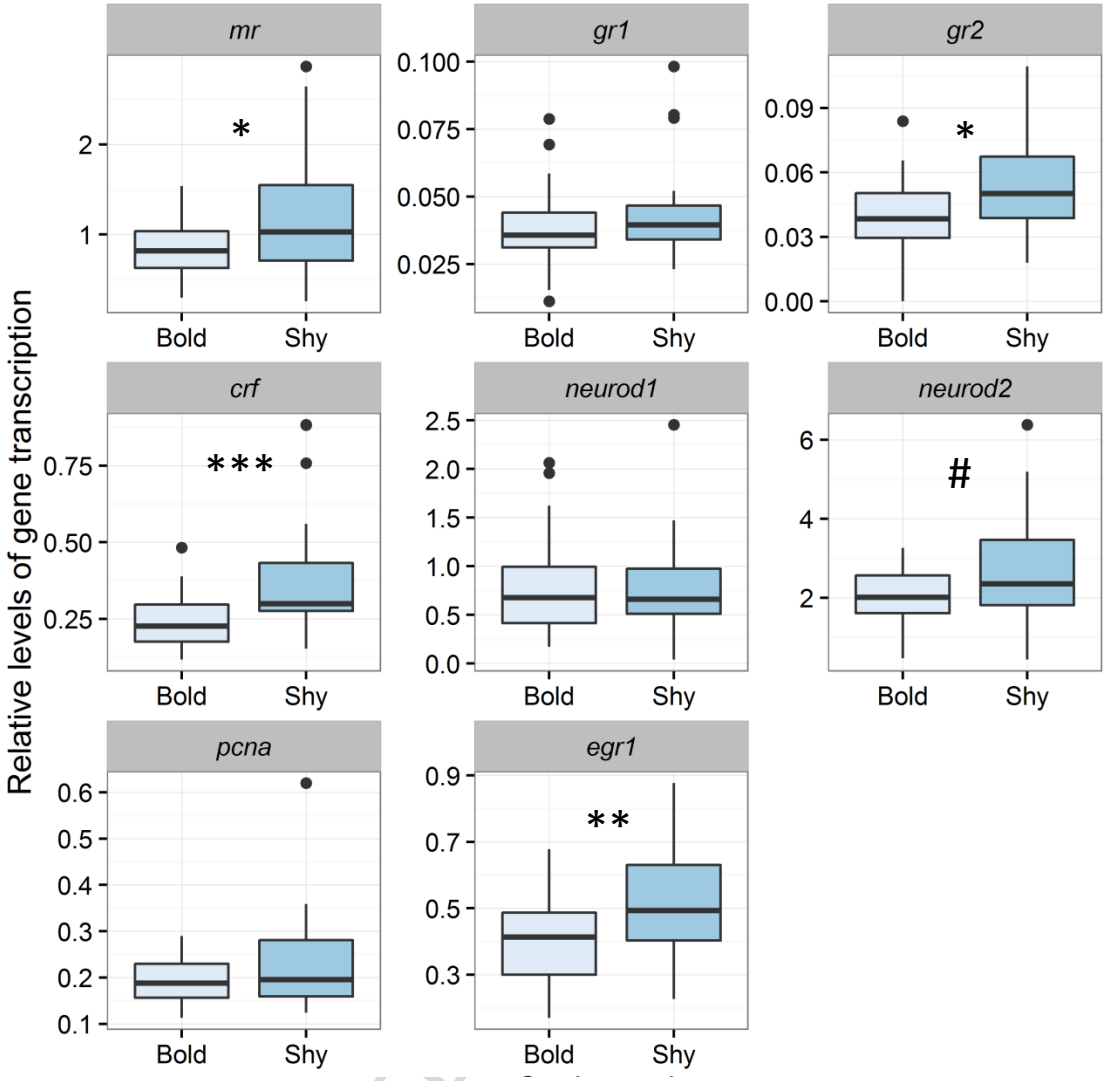

Figure 4. Relative levels of gene transcription of the *mineralocorticoid receptor* (*mr*), *glucocorticoid receptor* 1 (*gr1*), *glucocorticoid receptor* 2 (*gr2*), *corticotropin-releasing factor* (*crf*), *neurogenic differentiation factors* (*neurod1* and *neurod2*), *proliferating cell nuclear antigen* (*pcna*), and *early growth response* (*egr1*) relative to reference genes (*l13* and *eef1-alpha*) in the brains of bold and shy fish categories assigned after the open field test (OFT). Wilcoxon rank sum test (Student's t-test for *egr1*): #: $p < 0.10$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. Note that for *gr2*, 6 cDNA samples were lost and are not presented.

3.6.Principal component analysis (PCA)

On one hand, PCA axis 1, which explained 52 % of the data variability observed, was mainly driven by the time spent in shelter, the distance travelled and the number of transitions between shelter and open field arena during the OFT (**Table 3**). On the other hand, PCA axis 2 was mainly driven by the latency to exit the shelter and the number of transitions between shelter and open field arena during the OFT and explained 28 % of the data variability observed (**Table 3**). PCA axis 1 was thus rather representative of activity in the OFT with individuals displaying high values for PCA axis 1 being more active in the OFT than individuals displaying low values. PCA axis 2 was rather representative of coping styles and individuals displaying high values for PCA axis 2 scores were shyer and moved more between shelter and open field arena than individuals with low values which were bolder and moved less between the two compartments of the OFT. Because of PCA axis 2 being more representative of coping styles, further analyses were performed on PCA axis 2 scores.

Table 3. Loading of behavioral variables (Latency to exit shelter, distance travelled, time spent in shelter and number of transitions between shelter and open field arena during the open field test) for the two PCA axes. Values in bold are the main drivers of the related axis.

Variable contribution	PCA axis 1	PCA axis 2
Latency to exit shelter	0.02	**0.90**
Distance travelled	**0.94**	-0.18
Time spent in shelter	**-0.95**	0.13
Number of transitions	**0.56**	**0.50**
Variability explained (%)	52	28

Regarding monoamines concentration in the brain, concentration of DA was negatively correlated with PCA axis 2 ($F = 5.25$, $p = 0.03$, $R^2 = 0.12$), *i.e.*, bolder fish displayed higher level of DA in the brain but there was no correlation for DOPAC or DOPAC/DA ratio with PCA axis 2 ($F = 0.98$, $p = 0.33$ and $F = 0.002$, $p = 0.96$ respectively). Moreover, 5HT and 5HIAA also were negatively correlated with PCA axis 2 scores ($F = 6.10$, $p = 0.02$, $R^2 = 0.14$ and $F = 7.96$, $p = 0.008$, $R^2 = 0.18$ respectively) but there was no correlations between 5HIAA/5HT ratio and PCA axis 2 ($F = 0.39$, $p = 0.54$). Finally there was no correlation between concentration of NE in the brain and PCA axis 2 scores ($F = 1.4$ $p = 0.25$, **Figure 5**).

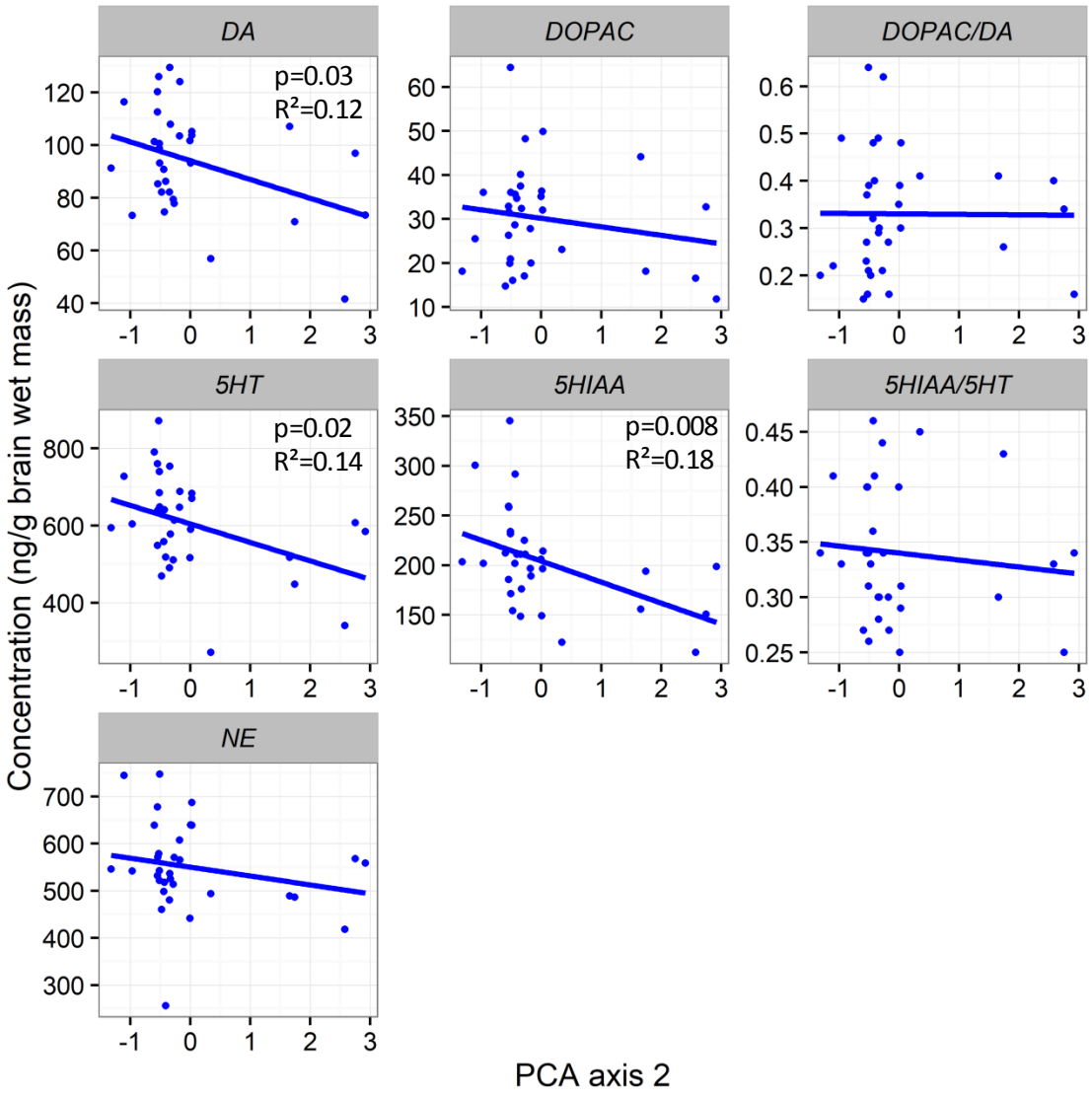

Figure 5. Concentration (ng/g brain wet mass) of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), the DOPAC/DA turnover ratio, serotonin (5HT), 5-hydroxyindoleacetic acid (5HIAA), the 5HIAA/5HT turnover ratio, and norepinephrine (NE) in the brain in relation to individual PCA axis 2 scores. P-value (p) and the associated R^2 are indicated for each significant linear regression.

Concerning gene transcription in the brain, there was no correlation between the investigated genes and PCA axis 2 scores except a negative trend between relative level of *pcna* and PCA axis 2 scores ($F = 3.99$, $p = 0.07$, $R^2 = 0.08$). All statistical details concerning correlations between the relative levels of other genes are given in the text of the **Supplementary Material** and illustrated in **Figure S1**.

4. Discussion

Here, we evaluated the boldness of European sea bass using two different challenges, which were group risk taking test (GRT) and open field test (OFT) at 227 and 749 dpf, respectively. The proportion of bold/shy fish did not differ between the two tests (approximately 60% of the fish were categorized as bold). The proportion of bold/shy fish however differed from a previous study in European sea bass in which ~80% were identified as shy using a group risk-taking test [56]. The high proportion of bold fish identified in our study may be attributable to small differences with their protocol and/or the level of fish domestication which could affect boldness [6]. We did not find any clear behavioral consistency in boldness across context between the GRT and OFT. Indeed, only 50% of fish with shy behavior in the GRT were also categorized as shy in the OFT (522 days between the two tests). The consistency of boldness over time was previously reported in European sea bass when consecutive GRTs were performed 29 days apart [4]. Boldness, as well as other personality traits, is known to be consistent over a short period of time, but can evolve during the life of the animal [70].

Moreover, boldness is influenced by many external factors, both biotic and abiotic [71-74]. Abiotic factors, such as temperature, physico-chemical characteristics of the water, or food supply, were kept constant during our experiment. However, some other factors could explain the absence of a clear boldness consistency between the GRT and OFT. The difference in social context between the two tests (group *vs* isolation) could be one and could partly explain the discrepancy between the results, regardless of the time window that separated the two behavioral experiments [75]. Social context is indeed very important in shaping personality traits in fish [76] and may have affected our capacity to evaluate boldness in single fish in a social species, such as sea bass, as already mentioned by Ferrari and colleagues [4].

Nevertheless, the fish expressed two divergent behavioral responses during the OFT. The majority of fish (58 %) expressed bold trait, with a high level of activity in the OFT arena, whereas shy fish expressed freezing behavior in the shelter. These two different behavioral responses have been largely described in the literature as typical proactive and reactive coping style characteristics [2, 5, 77].

Differential stress regulation of the HPI axis and the brain-chromaffin axis are often associated with the opposite behavioral responses expressed by proactive and reactive individuals [1, 2, 35, 78]. Here, the signal from the dopaminergic system in the whole brain was similar between bold and shy fish. The concentrations of DA and DOPAC and the DA/DOPAC turnover ratio were indeed identical between our two groups, whereas dopaminergic system signaling was found to be different between bold and shy fish in different brain parts such as optic tectum [26, 79]. Possible specific differences between bold and shy fish could also be masked by the fact that the analyses were performed on whole brain [26, 80]. The boldest fish screened in the OFT nevertheless displayed higher DA concentration in the whole brain compared to the less bold ones. This is consistent with previous studies suggesting that dopaminergic system may trigger active behavioral

responses, including bolder behavior [81-83]. Nevertheless, it is important to note that our study was performed on the whole brain, therefore we cannot conclude that the differences between bold and shy fish are representative of what would be measured in region-specific areas. Concerning the serotonergic system, the 5HT/5HIAA turnover ratio was lower in shy than bold fish following the OFT but the boldest fish displayed both higher levels of 5HT and 5HIAA in the brain than the less bold ones. Similar differences in 5HIAA have been previously observed in the hypothalamus and telencephalon of rainbow trout (*Oncorhynchus mykiss*) following an acute stressor [26]; fish with a more reactive coping style showed lower levels of serotonergic activity upon exposure to stress. This supports the view of the shy and bold groups established in the OFT as exhibiting different coping styles, as differences in the serotonergic system could be involved in the determination of the individual coping style [84]. Another interpretation is that the differential behavior observed (exploring *vs* freezing in the shelter) might have driven the difference in the serotonergic response. In this case, the bold fish were more active and left the shelter and thus exposed themselves out in the open area, which might be more stressful for them, leading to higher 5HT activity. This alternative interpretation has been considered by Koolhaas and colleagues [85] who hypothesized that "it is likely that differential neuroendocrine characteristics are mainly a consequence rather that the cause of behavioral differentiation". Finally, the noradrenergic system is generally less well-documented than the serotonergic and dopaminergic systems in relation to coping styles. However, the higher NE concentrations we measured in the whole brain of shy fish correlate well with results found in the brainstem, telencephalon and optic tectum of rainbow trout following confinement stress [26, 80].

Concerning the HPI axis, plasma cortisol concentrations measured in our study were higher than those previously found in European sea bass [86, 87]. It is likely that the social isolation during the OFT is a very strong stressor for European sea bass, as was shown for rainbow

trout [88]. Further, there was no difference in the cortisol response between bold and shy fish categorized using the OFT. This may seem surprising, as high cortisol levels have been shown to be predictive of the reactive coping style in sea bass in another context [4]. Nevertheless, it cannot be excluded that the lack of difference may be due to the fact that, once in the OFT, bold fish take more risk and are further stressed by exploring the open field arena whereas shy fish stay in the shelter resulting in different physiological status when blood sample was taken. Moreover, such an absence of a difference in the peak cortisol response was previously reported following an acute stressor in various fish species [3, 78], but divergences appeared during the recovery phase [3]. The transcription in the whole brain of *gr2*, *mr* and *crf*, which involved in the regulation of the HPI axis, was higher in shy than bold fish, whereas *gr1* transcription did not differ between the two groups. Elevated *gr2*, *mr* or *crf* transcription in shy fish has also been reported in other fish species and contexts [80, 89-91]. For example, HR rainbow trout (considered as shy) displayed higher levels of *crf* than LR (considered as bold) in the forebrain of rainbow trout following confinement stress [80]. These effects were shown to be modulated by binding proteins reducing the activity of Crf but increasing its longevity [92, 93]. In addition to its effects on the regulation of the HPI axis, Crf can interact with neurotransmission and modulate behavior [94-97]. Overall, Crf interacts with serotonergic and noradrenergic pathways, resulting in an increase in locomotor behavior, including non-ambulatory behavior, and has been reported to cause anxiety [95]. In the present study, shy fish (which expressed higher levels of *crf* than bold fish) expressed a lower turnover of serotonergic activity and higher NE concentrations. Shy fish also displayed a freezing behavior in the shelter during the OFT. This echoes a previous study showing that intraventricular Crf injections in rats increase the anxiety state of the animal, as well as the turnover ratio between NE and its metabolite DHPG in the brain-stem locus coeruleus [98]. More recently, it was reported in rainbow trout that Crf-injected fish have higher 5HT and 5

HIAA levels in various brain regions (*i.e.*, the subpallium, raphe, and preoptic hypothalamus) and/or express head-shaking behavior, which is related to anxiety [99, 100]. Interactions between Crf and both the serotonergic and the noradrenergic system appear promising to further explain the divergent phenotypes observed in fish coping styles [95, 99, 100].

Recent studies suggest that stress axis regulation and neurogenesis are tightly linked [45-47]. For example, Sadoul and colleagues [45] have shown that high levels of *mr* and *gr* in the brain correlate with high levels of *pcna* and *neurod1* in several fish species, including the European sea bass. Here, we also detected a strong and significant correlation (at the individual level) between genes involved in the stress response and those involved in neurogenesis (**supplementary material**), confirming the results of Sadoul and colleagues [45]. In the present study, we show that the expression of *egr1* and *neurod2*, which are important markers of neurogenesis involved in neural activation and differentiation, respectively [34, 48], were higher in the brains of shy fish than in those of bold fish (only a trend for *neurod2*). This higher propensity to display strong neurogenesis in shy fish is suggested in the literature [34, 89, 90, 101] and may be important for learning [34]. This is in accordance with previous results showing that shy fish are more proficient in learning and triggering self-feeders [60, 102].

Here, both monoamine concentrations and gene transcription analyses were performed on the whole brain, including multiple regions with specific functions and patterns of monoamine synthesis and gene transcription [26, 78, 103]. Significant effects were observed using the whole brain, and therefore we could expect even more significant differences between groups in specific areas of the brain. Microdissections or dissection of the different areas of the brain would have probably lead to more robust conclusions. A refined analysis would be necessary to better understand the differential pattern of responses in the different brain areas.

Overall, European sea bass characterized by two divergent behavioral phenotypes on the boldness axis, bold and shy, also display two distinct physiological responses which could be reflect the proactive and reactive differentiation, respectively. Altogether, our results suggest that the two coping styles display differences in the HPI axis, the serotonergic and noradrenergic system reactivity, and in neurogenesis. Further, and according to our results, the behavioral responses of a social animal to a challenge in isolation seem to be relevant markers of its physiological state and capacity to regulate and react to a stressor. More data are needed to verify whether such responses are specific to one time point or if they are indicators of a lifelong coping style.

Acknowledgements

We are grateful to Marie-Odile Vidal, François Ruelle, and Alain Vergnet for hatching and rearing the fish. We would also like to thank Mako Pegart for valuable help with the gene transcription analyses. SA received a PhD grant from ERANET ANIHWA WINFISH (ANR-14-ANWA-0008) and Ifremer and BS received a postdoctoral grant from ERANET COFASP SUSHIFISH (ANR-15-COFA-0002-01). We also thank the anonymous reviewers for helpful comments on the previous version of the manuscript.

References

[1] Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., et al. Coping styles in animals: current status in behavior and stress-physiology. Neuroscience & Biobehavioral Reviews. 1999,23:925-35.

[2] Øverli, Ø., Sørensen, C., Pulman, K. G., Pottinger, T. G., Korzan, W., Summers, C. H., et al. Evolutionary background for stress-coping styles: relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. Neurosci Biobehav Rev. 2007,31:396-412.

[3] Tudorache, C., Schaaf, M. J., Slabbekoorn, H. Covariation between behaviour and physiology indicators of coping style in zebrafish (*Danio rerio*). J Endocrinol. 2013,219:251-8.

[4] Ferrari, S., Millot, S., Leguay, D., Chatain, B., Bégout, M. L. Consistency in European seabass coping styles: A life-history approach. Applied Animal Behaviour Science. 2015,167:74-88.

[5] Castanheira, M. F., Conceição, L. E. C., Millot, S., Rey, S., Bégout, M. L., Damsård, B., et al. Coping styles in farmed fish: consequences for aquaculture. Reviews in Aquaculture. 2017,9:23-41.
[6] Millot, S., Bégout, M.-L., Chatain, B. Exploration behaviour and flight response toward a stimulus in three sea bass strains (*Dicentrarchus labrax* L.). Applied Animal Behaviour Science. 2009,119:108-14.
[7] Øverli, Ø., Korzan, W. J., Larson, E. T., Winberg, S., Lepage, O., Pottinger, T. G., et al. Behavioral and neuroendocrine correlates of displaced aggression in trout. Horm Behav. 2004,45:324-9.
[8] Huntingford, F. A., Andrew, G., Mackenzie, S., Morera, D., Coyle, S. M., Pilarczyk, M., et al. Coping strategies in a strongly schooling fish, the common carp Cyprinus carpio. J Fish Biol. 2010,76:1576-91.
[9] Geffroy, B., Sadoul, B., Bardonnet, A. Behavioural syndrome in juvenile eels and its ecological implications. Behaviour. 2015,152:147-66.
[10] Øverli, Ø., Sørensen, C., Nilsson, G. E. Behavioral indicators of stress-coping style in rainbow trout: Do males and females react differently to novelty? Physiology & Behavior. 2006,87:506-12.
[11] Killen, S. S., Marras, S., McKenzie, D. J. Fuel, fasting, fear: routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. Journal of Animal Ecology. 2011,80:1024-33.
[12] Rey, S., Boltana, S., Vargas, R., Roher, N., Mackenzie, S. Combining animal personalities with transcriptomics resolves individual variation within a wild-type zebrafish population and identifies underpinning molecular differences in brain function. Mol Ecol. 2013,22:6100-15.
[13] MacKenzie, S., Ribas, L., Pilarczyk, M., Capdevila, D. M., Kadri, S., Huntingford, F. A. Screening for coping style increases the power of gene expression studies. PloS one. 2009,4:e5314.
[14] Johansen, I. B., Sørensen, C., Sandvik, G. K., Nilsson, G. E., Höglund, E., Bakken, M., et al. Neural plasticity is affected by stress and heritable variation in stress coping style. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics. 2012,7:161-71.
[15] Silva, P. I. M., Martins, C. I. M., Engrola, S., Marino, G., Øverli, Ø., Conceição, L. E. C. Individual differences in cortisol levels and behaviour of Senegalese sole (*Solea senegalensis*) juveniles: Evidence for coping styles. Applied Animal Behaviour Science. 2010,124:75-81.
[16] Barton, B. A. Stress in finfish: past, present and future - a historical perspective. In: Fish Stress and Health in Aquaculture (Iwama, G.K., Pickering, A.D., Sumpter, J.P. and Schreck, C.B.), Cambridge University Press, Cambridge, U.K. 1997:1-33 pp.
[17] Gesto, M., Lopez-Patino, M. A., Hernandez, J., Soengas, J. L., Miguez, J. M. The response of brain serotonergic and dopaminergic systems to an acute stressor in rainbow trout: a time course study. Journal of Experimental Biology. 2013,216:4435-42.
[18] Sumpter, J. P. The endocrinology of stress. Fish Stress and Health in Aquaculture, Cambridge University Press, Cambridge. 1997,Society for Experimental Biology, Seminar series:62:95-118.
[19] Barton, B. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integ. and Comp. Biol. 2002,42:517-25.
[20] Wendelaar Bonga, S. E. The stress response in fish. Physiological Reviews. 1997,77:591-625.
[21] Reid, S. G., Bernier, N. J., Perry, S. F. The adrenergic stress response in fish: control of catecholamine storage and release. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology. 1998,120:1-27.

[22] Winberg, S., Nilsson, A., Hylland, P., Söderstöm, V., Nilsson, G. E. Serotonin as a regulator of hypothalamic-pituitary-interrenal activity in teleost fish. Neuroscience Letters. 1997,230:113-6.
[23] Gesto, M., Tintos, A., Soengas, J. L., Miguez, J. M. Effects of acute and prolonged naphthalene exposure on brain monoaminergic neurotransmitters in rainbow trout (Oncorhynchus mykiss). Comp Biochem Physiol C Toxicol Pharmacol. 2006,144:173-83.
[24] Gesto, M., Tintos, A., Soengas, J. L., Miguez, J. M. beta-Naphthoflavone and benzo(a)pyrene alter dopaminergic, noradrenergic, and serotonergic systems in brain and pituitary of rainbow trout (*Oncorhynchus mykiss*). Ecotoxicology and Environmental Safety. 2009,72:191-8.
[25] Gesto, M., Soengas, J. L., Miguez, J. M. Acute and prolonged stress responses of brain monoaminergic activity and plasma cortisol levels in rainbow trout are modified by PAHs (naphthalene, beta-naphthoflavone and benzo(a)pyrene) treatment. Aquatic Toxicology. 2008,86:341-51.
[26] Øverli, Ø., Pottinger, T. G., Carrick, T. R., Øverli, E., Winberg, S. Brain monoaminergic activity in rainbow trout selected for high and low stress responsiveness. Brain Behav Evolut. 2001,57:214-24.
[27] Winberg, S., Nilsson, G. r. E. Roles of brain monoamine neurotransmitters in agonistic behaviour and stress reactions, with particular reference to fish. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology. 1993,106:597-614.
[28] Winberg, S., Nilsson, G. E., Olsen, K. H. The Effect of Stress and Starvation on Brain-Serotonin Utilization in Arctic Charr (Salvelinus-Alpinus). Journal of Experimental Biology. 1992,165:229-39.
[29] Shannon, N. J., Gunnet, J. W., Moore, K. E. A Comparison of Biochemical Indexes of 5-Hydroxytryptaminergic Neuronal-Activity Following Electrical-Stimulation of the Dorsal Raphe Nucleus. Journal of Neurochemistry. 1986,47:958-65.
[30] Hoglund, E., Weltzien, F. A., Schjolden, J., Winberg, S., Ursin, H., Doving, K. B. Avoidance behavior and brain monoamines in fish. Brain research. 2005,1032:104-10.
[31] Brelin, D., Petersson, E., Winberg, S. Divergent stress coping styles in juvenile brown trout (*Salmo trutta*). Annals of the New York Academy of Sciences. 2005,1040:239-45.
[32] Winberg, S., Thornqvist, P. O. Role of brain serotonin in modulating fish behavior. Current Zoology. 2016,62:317-23.
[33] Øverli, Ø., Korzan, W. J., Hoglund, E., Winberg, S., Bollig, H., Watt, M., et al. Stress coping style predicts aggression and social dominance in rainbow trout. Horm Behav. 2004,45:235-41.
[34] Sørensen, C., Johansen, I. B., Øverli, Ø. Neural plasticity and stress coping in teleost fishes. Gen Comp Endocrinol. 2013,181:25-34.
[35] Vindas, M. A., Gorissen, M., Hoglund, E., Flik, G., Tronci, V., Damsgard, B., et al. How do individuals cope with stress? Behavioural, physiological and neuronal differences between proactive and reactive coping styles in fish. J Exp Biol. 2017,220:1524-32.
[36] Qu, J. P., Fletcher, Q. E., Reale, D., Li, W. J., Zhang, Y. M. Independence between coping style and stress reactivity in plateau pika. Physiology & Behavior. 2018,197:1-8.
[37] Sadoul, B., Vijayan, M. M. Stress and growth. Fish physiology. 2016,Biology of stress in fish, academic Press:167-205.
[38] Schreck, C. B., Tort, L. The concept of stress in fish. Fish physiology. 2016,Biology of stress in fish, academic Press:1-34.
[39] Kiilerich, P., Geffroy, B., Valotaire, C., Prunet, P. Endogenous regulation of 11-deoxycorticosterone (DOC) and corticosteroid receptors (CRs) during rainbow trout early development and the effects of corticosteroids on hatching. General and Comparative Endocrinology. 2018,265:22-30.

[40] Johansen, I. B., Sandvik, G. K., Nilsson, G. E., Bakken, M., Øverli, Ø. Cortisol receptor expression differs in the brains of rainbow trout selected for divergent cortisol responses. Comparative biochemistry and physiology. Part D, Genomics & proteomics. 2011,6:126-32.
[41] Alderman, S. L., McGuire, A., Bernier, N. J., Vijayan, M. M. Central and peripheral glucocorticoid receptors are involved cortisol response to an acute stressor in rainbow trout. General and Comparative Endocrinology. 2012,176:79-85.
[42] Bernier, N. J., Flik, G., Klaren, P. H. M. Chapter 6 regulation and contribution of the corticotropic, melanotropic and thyrotropic axes to the stress response in fishes. Fish physiology, Academic press. 2009.
[43] Faught, E., Aluru, N., Vijayan, M. M. The molecular stress reponse. Fish physiology. 2016,Biology of stress in fish, academic Press:133-66.
[44] Doyon, C., Gilmour, K. M., Trudeau, V. L., Moon, T. W. Corticotropin-releasing factor and neuropeptide Y mRNA levels are elevated in the preoptic area of socially subordinate rainbow trout. General and Comparative Endocrinology. 2003,133:260-71.
[45] Sadoul, B., Alfonso, S., Bessa, E., Bouchareb, A., Blondeau-Bidet, E., Clair, P., et al. Enhanced brain expression of genes related to cell proliferation and neural differentiation is associated with cortisol receptor expression in fishes. Gen Comp Endocrinol. 2018,267:76-81.
[46] Sørensen, C., Bohlin, L. C., Øverli, Ø., Nilsson, G. E. Cortisol reduces cell proliferation in the telencephalon of rainbow trout (Oncorhynchus mykiss). Physiol Behav. 2011,102:518-23.
[47] Best, C., Kurrasch, D. M., Vijayan, M. M. Maternal cortisol stimulates neurogenesis and affects larval behaviour in zebrafish. Scientific reports. 2017,7:40905.
[48] Burmeister, S. S., Fernald, R. D. Evolutionary conservation of the egr-1 immediate-early gene response in a teleost. J Comp Neurol. 2005,481:220-32.
[49] Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V., Montiglio, P.-O. Personality and the emergence of the pace-of-life syndrome concept at the population level. Philosophical Transactions of the Royal Society B: Biological Sciences. 2010,365:4051-63.
[50] Geffroy, B., Bardonnet, A. Differential effects of behaviour, propensity to migrate and recruitment season on glass eels and elvers' growing performance. Ecology of Freshwater Fish. 2012,21:469-82.
[51] Martins, C. I. M., Schrama, J. W., Verreth, J. A. J. The consistency of individual differences in growth, feed efficiency and feeding behaviour African catfish *Clarias gariepinus* (Burchell 1822) housed individually. Aquaculture Research. 2005,36:1509-16.
[52] Martins, C. I. M., Trenovski, M., Schrama, J. W., Verreth, J. A. J. Comparison of feed intake behaviour and stress response in isolated and non-isolated African catfish. Journal of Fish Biology. 2006,69:629-36.
[53] FAO. The state of world fisheries and aquaculture.: Food and Organisation of the United Nations; 2012. p. 182 pp.
[54] Whitehead, P. J. P., Bauchot, M. L., Hureau, J. C., Nielsen, J., Tortonese, E. Fish of the North-eastern Atlantic and Mediterranean. UNESCO. 1986,2.
[55] Tveteras, R., Nystoyl, R. Fish production. 2011,Estimates & trends 2011–2012 Santiago, Chile.
[56] Ferrari, S., Horri, K., Allal, F., Vergnet, A., Benhaim, D., Vandeputte, M., et al. Heritability of boldness and hypoxia avoidance in European seabass, *Dicentrarchus labrax*. PloS one. 2016,11.
[57] Sih, A., Cote, J., Evans, M., Fogarty, S., Pruitt, J. Ecological implications of behavioural syndromes. Ecology Letters. 2012,15:278-89.
[58] Conrad, J. L., Weinersmith, K. L., Brodin, T., Saltz, J. B., Sih, A. Behavioural syndromes in fishes: a review with implications for ecology and fisheries management. Journal of Fish Biology. 2011,78:395-435.

[59] Chatain, B. Estimation et amélioration des performances zootechniques de l'élevage larvaire de *Dicentrarchus labrax* et de *Sparus auratus*. 1994.
[60] Benhaim, D., Akian, D. D., Ramos, M., Ferrari, S., Yao, K., Begout, M. L. Self-feeding behaviour and personality traits in tilapia: A comparative study between Oreochromis niloticus and Sarotherodon melanotheron. Applied Animal Behaviour Science. 2017,187:85-92.
[61] Sadoul, B., Geffroy, B. Measuring cortisol, the major stress hormone in fishes. Journal of Fish Biology. 2019,Accepted for publication.
[62] Geffroy, B., Guilbaud, F., Amilhat, E., Beaulaton, L., Vignon, M., Huchet, E., et al. Sexually dimorphic gene expressions in eels: useful markers for early sex assessment in a conservation context. Scientific Reports. 2016,6.
[63] Pavlidis, M., Karantzali, E., Fanouraki, E., Barsakis, C., Kollias, S., Papandroulakis, N. Onset of the primary stress in European sea bass Dicentrarhus labrax, as indicated by whole body cortisol in relation to glucocorticoid receptor during early development. Aquaculture. 2011,315:125-30.
[64] Crespo, B., Gomez, A., Mazon, M. J., Carrillo, M., Zanuy, S. Isolation and characterization of Ff1 and Gsdf family genes in European sea bass and identification of early gonadal markers of precocious puberty in males. Gen Comp Endocrinol. 2013,191:155-67.
[65] Radonic, A., Thulke, S., Mackay, I. M., Landt, O., Siegert, W., Nitsche, A. Guideline to reference gene selection for quantitative real-time PCR. Biochemical and Biophysical Research Communications. 2004,313:856-62.
[66] Bland, F., McIntosh, R., Bain, N., Snow, M. Development and validation of a range of endogenous controls to support the implementation of practical Taqman real-time PCR-based surveillance for fish diseases within aquaculture. J Fish Dis. 2012,35:447-54.
[67] Pfaffl, M. W. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001,29:e45.
[68] team, R. C. R: A language and environment for statistical computing. . R Foundation for Statistical Computing, Vienna, Austria. 2013.
[69] Dray, S., Dufour, A. B. The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software. 2007,22:1-20.
[70] Polverino, G., Cigliano, C., Nakayama, S., Mehner, T. Emergence and development of personality over the ontogeny of fish in absence of environmental stress factors. Behavioral Ecology and Sociobiology. 2016,70:2027-37.
[71] Brown, C., Burgess, F., Braithwaite, V. A. Heritable and experiential effects on boldness in a tropical poeciliid. Behavioral Ecology and Sociobiology. 2007,62:237-43.
[72] Thomson, J. S., Watts, P. C., Pottinger, T. G., Sneddon, L. U. Plasticity of boldness in rainbow trout, Oncorhynchus mykiss: do hunger and predation influence risk-taking behaviour? Hormones and Behavior. 2012,61:750-7.
[73] Frost, A. J., Winrow-Giffen, A., Ashley, P. J., Sneddon, L. U. Plasticity in animal personality traits: does prior experience alter the degree of boldness? P Roy Soc B-Biol Sci. 2007,274:333-9.
[74] Ferrari, S., Leguay, D., Vergnet, A., Vidal, M. O., Chatain, B., Begout, M. L. Unpredictability in food supply during early life influences growth and boldness in European seabass, Dicentrarchus labrax (vol 180, pg 147, 2016). Applied Animal Behaviour Science. 2016,185:152-.
[75] Webster, M. M., Ward, A. J. W., Hart, P. J. B. Boldness is influenced by social context in threespine sticklebacks (Gasterosteus aculeatus). Behaviour. 2007,144:351-71.
[76] Geffroy, B., Bru, N., Dossou-Gbete, S., Tentelier, C., Bardonnet, A. The link between social network density and rank-order consistency of aggressiveness in juvenile eels. Behavioral Ecology and Sociobiology. 2014,68:1073-83.

[77] Øverli, Ø., Sørensen, C., Nilsson, G. E. Behavioral indicators of stress-coping style in rainbow trout: Do males and females react differently to novelty? Physiol Behav. 2006,87:506-12.
[78] Vindas, M. A., Magnhagen, C., Brannas, E., Øverli, Ø., Winberg, S., Nilsson, J., et al. Brain cortisol receptor expression differs in Arctic charr displaying opposite coping styles. Physiol Behav. 2017,177:161-8.
[79] Koolhaas, J. M., de Boer, S. F., Buwalda, B., van Reenen, K. Individual variation in coping with stress: A multidimensional approach of ultimate and proximate mechanisms. Brain Behav Evolut. 2007,70:218-26.
[80] Backstrom, T., Schjolden, J., Øverli, Ø., Thornqvist, P. O., Winberg, S. Stress effects on AVT and CRF systems in two strains of rainbow trout (Oncorhynchus mykiss) divergent in stress responsiveness. Horm Behav. 2011,59:180-6.
[81] Winberg, S., Nilsson, G. E., Olsen, K. H. Social Rank and Brain Levels of Monoamines and Monoamine Metabolites in Arctic-Charr, Salvelinus-Alpinus (L). J Comp Physiol A. 1991,168:241-6.
[82] Winberg, S., Nilsson, G. E. Induction of Social-Dominance by L-Dopa Treatment in Arctic Charr. Neuroreport. 1992,3:243-6.
[83] Thornqvist, P. O., McCarrick, S., Ericsson, M., Roman, E., Winberg, S. Bold zebrafish (Danio rerio) express higher levels of delta opioid and dopamine D2 receptors in the brain compared to shy fish. Behav Brain Res. 2018.
[84] Puglisi-Allegra, S., Andolina, D. Serotonin and stress coping. Behavioural Brain Research. 2015,277:58-67.
[85] Koolhaas, J. M., de Boer, S. F., Coppens, C. M., Buwalda, B. Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. Frontiers in neuroendocrinology. 2010,31:307-21.
[86] Rotllant, J., Ruane, N. M., Caballero, M. J., Montero, D., Tort, L. Response to confinement in sea bass (Dicentrarchus labrax) is characterised by an increased biosynthetic capacity of interrenal tissue with no effect on ACTH sensitivity. Comparative biochemistry and physiology. Part A, Molecular & integrative physiology. 2003,136:613-20.
[87] Samaras, A., Dimitroglou, A., Sarropoulou, E., Papaharisis, L., Kottaras, L., Pavlidis, M. Repeatability of cortisol stress response in the European sea bass (Dicentrarchus labrax) and transcription differences between individuals with divergent responses. Scientific reports. 2016,6:34858.
[88] Bernier, N. J., Alderman, S. L., Bristow, E. N. Heads or tails? Stressor-specific expression of corticotropin-eleasing factor and urotensin I in the preoptic area and caudal neurosecretory system of rainbow trout. Journal of Endocrinology. 2008,196:637-48.
[89] Geffroy, B., Sadoul, B., Bouchareb, A., Prigent, S., Bourdineaud, J. P., Gonzalez-Rey, M., et al. Nature-Based Tourism Elicits a Phenotypic Shift in the Coping Abilities of Fish. Front Physiol. 2018,9:13.
[90] van den Bos, R., Mes, W., Galligani, P., Heil, A., Zethof, J., Flik, G., et al. Further characterisation of differences between TL and AB zebrafish (Danio rerio): Gene expression, physiology and behaviour at day 5 of the larval stage. PloS one. 2017,12.
[91] Summers, T. R., Summers, T. L., Carpenter, R. E., Smith, J. P., Young, S. L., Meyerink, B., et al. Learning and CRF-Induced Indecision during Escape and Submission in Rainbow Trout during Socially Aggressive Interactions in the Stress-Alternatives Model. Frontiers in neuroscience. 2017,11.
[92] Flik, G., Klaren, P. H. M., Van den Burg, E. H., Metz, J. R., Huising, M. O. CRF and stress in fish. General and Comparative Endocrinology. 2006,146:36-44.

[93] Samaras, A., Santo, C. E., Papandroulakis, N., Mitrizakis, N., Pavlidis, M., Hoglund, E., et al. Allostatic Load and Stress Physiology in European Seabass (Dicentrarchus labrax L.) and Gilthead Seabream (Sparus aurata L.). Front Endocrinol. 2018,9.
[94] Clements, S., Moore, F. L., Schreck, C. B. Evidence that acute serotonergic activation potentiates the locomotor-stimulating effects of corticotropin-releasing hormone in juvenile chinook salmon (*Oncorhynchus tshawytscha*). Hormones and Behavior. 2003,43:214-21.
[95] Lowry, C. A., Moore, F. L. Regulation of behavioral responses by corticotropin-releasing factor. General and Comparative Endocrinology. 2006,146:19-27.
[96] Carpenter, R. E., Korzan, W. J., Bockholt, C., Watt, M. J., Forster, G. L., Renner, K. J., et al. Corticotropin Releasing Factor Influences Aggression and Monoamines: Modulation of Attacks and Retreats. Neuroscience. 2009,158:412-25.
[97] Backstrom, T., Winberg, S. Serotonin Coordinates Responses to Social Stress-What We Can Learn from Fish. Frontiers in neuroscience. 2017,11.
[98] Butler, P. D., Weiss, J. M., Stout, J. C., Nemeroff, C. B. Corticotropin-Releasing Factor Produces Fear-Enhancing and Behavioral Activating Effects Following Infusion into the Locus Coeruleus. Journal of Neuroscience. 1990,10:176-83.
[99] Backstrom, T., Pettersson, A., Johansson, V., Winberg, S. CRF and urotensin I effects on aggression and anxiety-like behavior in rainbow trout. J Exp Biol. 2011,214:907-14.
[100] Carpenter, R. E., Watt, M. J., Forster, G. L., Øverli, Ø., Bockholt, C., Renner, K. I., et al. Corticotropin releasing factor induces anxiogenic locomotion in trout and alters serotonergic and dopaminergic activity. Hormones and Behavior. 2007,52:600-11.
[101] Øverli, Ø., Sørensen, C. On the Role of Neurogenesis and Neural Plasticity in the Evolution of Animal Personalities and Stress Coping Styles. Brain Behav Evolut. 2016,87:167-74.
[102] Ferrari, S., Benhaim, D., Colchen, T., Chatain, B., Begout, M. L. First links between self-feeding behaviour and personality traits in European seabass, Dicentrarchus labrax. Applied Animal Behaviour Science. 2014,161:131-41.
[103] Vindas, M. A., Fokos, S., Pavlidis, M., Hoglund, E., Dionysopoulou, S., Ebbesson, L. O. E., et al. Early life stress induces long-term changes in limbic areas of a teleost fish: the role of catecholamine systems in stress coping. Scientific Reports. 2018,8.

Highlights

- Boldness is not consistent when characterized using different types of test over an interval period of 16 months (group *vs.* individual tests).
- After open field test, plasma cortisol concentration is similar between bold and shy fish.
- Shy fish, however, display higher level of transcription of *mr*, *gr2*, *crf*, *egr1* and *neurod2* (trend) and also higher level of norepinephrine and lower turnover ratio between serotonin and its main metabolite (hydroxyindoleacetic acid) in the whole brain.