



Contrasting neurochemical and behavioral profiles reflects stress coping styles but not stress responsiveness in farmed gilthead seabream (*Sparus aurata*)

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ABSTRACT

In fish, as well as in other vertebrates, contrasting suites of physiological and behavioral traits, or coping styles, are often shown in response to stressors. However, the magnitude of the response (i.e. stress responsiveness) has been suggested to be independent of stress coping style. One central neurotransmitter that has been associated with both stress responsiveness and differences in stress coping styles is serotonin (5-hydroxytryptamine, 5-HT).

In this study, we investigated to what extent stress responsiveness reflects differences in stress coping, and the potential involvement of the 5-HT system in mediating such differences in farmed Gilthead seabream. Initially, fish were classified as proactive or reactive based on their behavioural response to net restraint. Following 1.5 months, fish classified as proactive still showed a higher number of escape attempts and spent longer time escaping than those classified as reactive. These differences were reflected in a generally higher brain stem 5-HT concentration and a lower telencephalic 5-HT activity, i.e. the ratio of 5-hydroxyindoleacetic acid (5-HIAA) to 5-HT, in proactive fish. Independent of stress coping styles, stress responsiveness was reflected in elevated 5-HIAA concentrations and 5-HIAA/5-HT ratios in telencephalon and brain stem together with increased plasma cortisol concentrations at 0.5 and 2 h following the last net restraint. The current results show that 5-HT signaling can reflect different behavioural output to a challenge which are independent of neuroendocrine responses to stress and lend support to the hypothesis that stress coping styles can be independent of stress responsiveness.

1. Introduction

Intra-specific divergence in behavioral profiles appears to be an evolutionary old phenomenon that has been reported in a broad spectrum of animals (see reviews by [1–4]). These individual behavioral differences are often referred to as animal personalities [1,6–8], behavioral syndromes [4], temperaments [9–11] or stress coping styles [2,3,12]. Personality or temperament have been defined as consistency of at least one behavioral trait [1], while behavioural syndromes is often used to describe correlated suites of behavioural traits [4]. Stress coping styles, in addition to being defined by consistent sets of behavioural traits are also characterized by consistent and divergent physiological traits displayed in response to stress [3]. Two broad patterns, the proactive and reactive stress coping styles, have been reported in a

number of animals, including teleost fish [5]. Animals displaying a proactive stress coping style generally show a fight-flight response and tend to be more aggressive and bolder than reactive individuals in response to challenges [3,13]. In contrast, characteristics of the reactive stress coping style includes a freeze-hide response (immobility), being non-aggressive and shy [3,13]. Moreover, there are several studies showing that proactive individuals respond to stress by a modest elevation of plasma cortisol concentrations but a pronounced increase in plasma catecholamines whereas reactive animals show the opposite pattern [3,13–16]. However, stress responsiveness (i.e. the magnitude of the neuroendocrine stress response) and type of response (i.e. proactive and reactive stress coping styles) have been suggested to represent two independent dimensions, forming an animal trait characteristic (for references see [2]). This is supported by studies

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demonstrating that individuals can show proactive behavioral characteristics independently of these physiological aspects of stress responsiveness [17–20]. Still, several studies demonstrate that individual variation in brain neurotransmission underlying contrasting stress coping styles are also involved in modulating the neuroendocrine stress response [2,5,12,14].

In fish, as well as in other vertebrates, behavioral and physiological responses are to a large degree linked by common control mechanisms in the brain. For example, divergent stress coping styles appears to be related to differences in brain serotonin (5-hydroxytryptamine, 5-HT) functions [3,5,13,14]. In addition, the 5-HTergic system is activated by stress and interacts with the hypothalamic-pituitary-adrenal (HPA) (in mammals) /hypothalamic-pituitary-interrenal (HPI) (in fish) axis activity. Following this, there is a general positive relationship between 5-HT release/production in the brain and HPA/I axis activity [21–24].

In general, comparative models have proven to be very useful in providing knowledge on fundamental principles of nervous system organization [25]. For instance, the high/low responsive (HR/LR) trout model has uncovered common evolutionary roots of behavioral and neuroendocrine trait associations underlying differences between the proactive and reactive stress coping styles within the vertebrate lineage [5]. In this model, the LR line, selected for low post stress plasma cortisol, show behavioral traits reminiscent to the proactive stress coping style, while the HR line, selected for high post stress plasma cortisol, show generally reactive traits. According to the general positive relationship between 5-HT and HPA/I axis activity, a higher 5-HT release or production in HR trout could be expected in this trout line. However, the opposite has been reported [26,27]. This made Øverli et al. [27] hypothesize that 5-HT is more related to the behavioral contrast than differences in HPI axis reactivity in the LR/HR model. In another fish model, the gilthead sea bream (*Sparus aurata*), Castanheira et al. [28] showed that the behavioral response to net restraint was related to the typical behavioral characteristics of stress coping styles, as described in other species. However, these behavioral traits were not related to post stress plasma cortisol, and it was suggested that behavioral response to net restraint only reflects stress coping styles and not stress reactivity in this species. Thus potentially, studies of the central mechanisms associated with the divergent behavioral responses to net restraint in seabass can provide knowledge on the role of 5-HT shaping the behavioural profiles associated with proactive and reactive coping styles.

The aims of the current study were to investigate to what extent differences in 5-HT brain neurochemistry is associated with contrasting stress coping styles and stress responsiveness in farmed seabream. To this end, pre- and post-stress plasma cortisol and brain 5-HT neurochemistry were compared between seabream previously characterized as proactive or reactive by net restraint test.

2. Materials and methods

2.1. Experimental animals

The experimental fish were obtained from a seabream producer (MARESA Mariscos de Esteros SA, Huelva, Spain) and were kept in stock groups until the start of the experiment in fiberglass stock tanks (500 L) under standard rearing conditions (for further details see Morales, 1983). Two weeks before the experiment started, all fish were individually PIT-tagged (Trovan®, Netherlands) and tagged with visible elastomers. At the start of the experiment juveniles of Gilthead seabream (*Sparus aurata*) used in this study weighed 49.14 ± 7.9 g (mean \pm SD).

2.2. Experimental facilities

Prior to the experiments, fish were submitted to a stress coping style screening test (detailed in Section 2.3.) and placed in 6 tanks (70 L) so

that each tank was housing 36 individuals with mixed coping styles and reared at standard conditions for 1.5 months.

Throughout the experimental period fish were kept on a natural photoperiod (37° 0' 22.35" N 7° 58' 3.35" W), with an average water temperature of 19.8 ± 1.1 °C, a salinity of 33.9 ± 2.1 ‰ and a dissolved oxygen concentration of $98.5 \pm 2.8\%$. Water quality parameters were checked daily. Fish were fed 2% BW day⁻¹, using automatic feeders, with a commercial diet (AquaGold 3 mm, Sorgal SA, Portugal; 44% crude protein, 14% crude fat, 8% ash, 2.5% crude fibres, 1.0% phosphorus) during all experimental procedures.

The experiment was conducted in accordance with the Guidelines of the European Union Council (86/609/EU) and Portuguese legislation for the use of laboratory animals. The experimental protocol was approved by the ethics committee from the Veterinary Medicines Directorate, the Portuguese competent authority for the protection of animals, Ministry of Agriculture, Rural Development and Fisheries, Portugal. Permit number 0420/000/000-n.99-09/11/2009.

2.3. Stress coping style screening

The tagged seabream juveniles ($n = 216$; 49.14 ± 7.9 g; mean \pm SD) were subjected to a net restraining test in order to determine their coping style (following previous described methodology by Castanheira et al., 2013). Briefly, each fish is restrained in a scoop net and exposed to air for 1 min. Fish behavior was video recorded (MicroVideo™ camera MCV2120-WP-LED, Canada) for later quantification of: i) latency to escape (time in seconds taken by each fish to show an escape attempt; i.e. an elevation of the body from the net); ii) number of escape attempts and iii) total time in seconds from first to last escape attempt. Fish behavior during restraining were analyzed using a stopwatch and collapsed into first principal component scores using Principal Components Analysis (PCA) (Table 1; for details of statistical analyses see Section 2.5). Individuals presenting a high latency to escape, low number of escape attempts and shorter total time escaping were characterized by a low score and identified as reactive fish, i.e. having a principal compound score less than -0.5 , Fig. 1. On opposite, individuals presenting a lower latency to escape, high number of escape attempts and spent longer time escaping were characterized by a high score and identified as proactive stress copers i.e. having a principal compound score over 0.5 (based on Castanheira et al., 2013). Immediately after the restraining test all individuals were placed back into their respective holding tanks. Following statistical analyses fish were categorized into proactive, reactive or intermediate coping styles, which were randomly mixed ($n = 36$; 12 individuals per style) in 6 tanks (100 L) and left undisturbed for 1.5 month before sampling.

2.4. Sampling protocol

After 1.5 months the same individuals were exposed to the same test as above (net restraining test for one min) thereafter transferred back to the rearing tanks. There were two sampling points 30 min and 2 h after the net restraining test. Control fish were immediately caught and killed with an overdose of 2-phenoxyethanol. They did not experience a net restraining test. Immediately after collection, individuals were killed

Table 1

Behavioral parameters quantified during a 5 min net restraint analysed with a principal compound analysis (PCA). These parameters were collapsed into a principal compound (PC 1), explaining 65% of the total variance.

Behavioral parameter	Mean (\pm SD)	Min.	Max.	Loading for PC 1
Latency to escape (sec)	14 ± 14	1	60	-0.77
Number of escapes attempts	11 ± 6	0	31	0.87
Total escaping time (sec)	6 ± 5	0	45	0.78

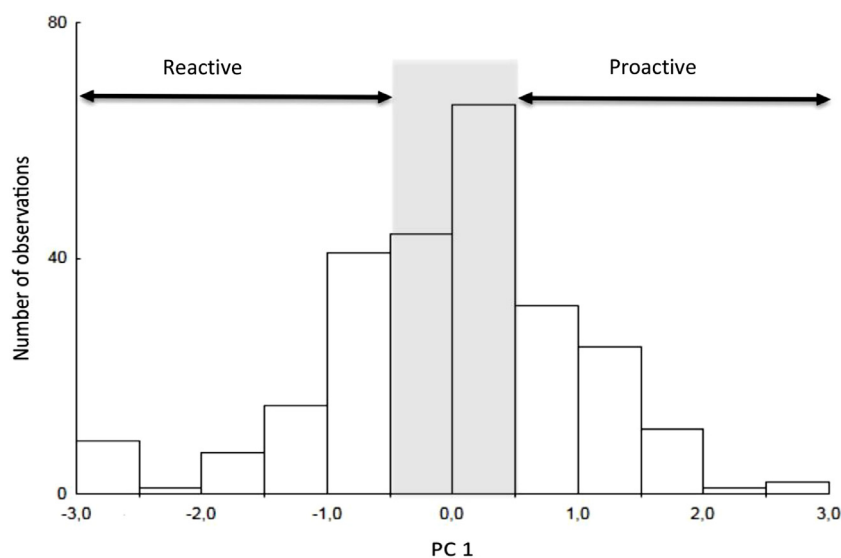


Fig. 1. Distribution of behavioral responses to net restraint in Gilthead seabream. Latency to escape, number of escape attempts and time spent showing escape behavior were collapsed into first principal component scores using Principal Components Analysis (PCA). Reactive fish, having a PC 1 score < -0.5 , were characterised by a low number of escape attempts, showed high latency to escape and spent shorter total time on escape behavior. In contrast, proactive fish, having a PC 1 score > 0.5 , showing high number of escape attempts, had a low latency to escape and spending longer time showing escape behavior.

with an overdose of 2-phenoxyethanol (Sigma-Aldrich), which rendered them completely motionless (no opercular movement) within 10 s of immersion. Collection of blood and brain samples were performed within 3 min. Blood samples were collected from caudal vein using heparinized syringes and centrifuged ($2000 \times g$ for 20 min) and plasma was separated and frozen in liquid nitrogen and stored in -80°C for posterior cortisol analysis. Brains were harvested and brain stem and telencephalon wrapped in individually labeled aluminium foil, frozen in liquid nitrogen and stored in -80°C for gene expression and monoamine analysis.

2.5. Plasma cortisol assay

Plasma cortisol levels were determined using a commercially available ELISA kit (RE52611, IBL International, Hamburg), with a sensitivity of 0.05 ng ml^{-1} and precision intra- and inter-assay coefficients of variation (CV) of 7.5 and 17%, respectively. This kit has been previously validated for seabream (López-Olmeda et al., 2009).

2.6. Brain neurochemistry

Sampled brainstems and telencephalons were weighed and homogenized using an ultrasonic disintegrator in a homogenizing reagent (4% perchloric acid (PCA) containing 0.2% EDTA and 40 ng ml^{-1} dihydroxy benzylamine hydroxide (DHBA) solution). After centrifuging the samples at 21,000 rpm for 10 min at 4°C , the supernatants were separated and analyzed by High-performance liquid chromatography (HPLC) with electrochemical detection to quantify the concentration of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5HIAA). The HPLC system consisted of a solvent delivery system (ESA, model 582, Bedford, MA, USA), an auto injector (Midas type 830, Spark Holland, Emmen, the Netherlands), a reverse phase column (4 mm 100 mm, DrMaisch, Reprosil-Pur C18, $3 \mu\text{m}$) and an ESA 5200 Coulochem II detector (Bedford, MA, USA) with two electrodes at -40 mV and $+320 \text{ mV}$. A conditioning electrode with potential of $+450 \text{ mV}$ was employed before the analytical electrodes to oxidize any contaminants. The ingredients in the mobile phase (HPLC buffer solution) were: 10.35 g l^{-1} monosodium dihydrogen phosphate (NaH_2PO_4), 0.3252 g l^{-1} sodium octyl sulphate (SOS), 0.0037 g l^{-1} EDTA, 70.0 ml l^{-1} acetonitril, pH was adjusted to 3.1 by adding concentrated phosphoric acid (H_3PO_4), and MilliQ water was added until 5 l in total. Telencephalic 5-HT and 5-HIAA content were quantified by comparing them with standard solutions of known concentrations and corrected for recovery of the internal standard using HPLC software (Clarity™, DataApex Ltd., the Czech

Republic). 5-HT turnover were calculated by dividing the concentration of monoamine metabolite 5-HIAA by the 5-HT concentration.

2.8. Statistics

Results are expressed as mean \pm standard error of the mean (SEM). Two-way analysis of variance (ANOVA) with post stress time and coping style as independent factors was applied to investigate the effects on plasma cortisol, 5-HIAA and 5-HT concentrations, and 5-HIAA/5-HT ratios. The Tukey's HSD (honest significant difference) post hoc test was used for detection of significant ($P < 0.05$) differences between groups. The 5-HT/5-HIAA ratios were arcsin transformed, and 5-HIAA and 5-HT concentrations were log transformed to obtain normal distribution. Data was analyzed with STATISTICA v. 12 Dell Inc.

Behaviors quantified in the net restraint test (latency to escape, number of escape attempts and total time spent on escape attempts) were collapsed into first principal component scores (PC1) using Principal Components Analysis (PCA), with orthogonal rotation (varimax). A correlation matrix was used to check multicollinearity, i.e., to identify variables that did not correlate with any other variable, or correlate very highly ($r = 0.9$) with one or more other variables. The Kaiser–Meyer–Olkin (KMO) test for sample adequacy was greater than 0.5 and Bartlett's test of sphericity was significant, indicating that correlation between items was sufficiently robust for PCA. At the initial behavioral characterization, fish with a principal component score < -0.5 were classified as displaying a reactive coping style while fish having a principal component score > 0.5 were classified as having proactive coping style. The behavioral response to net restraint was investigated by second PCA at sampling 1.5 months later, and differences in principal component score between reactive and proactive copers were investigated by a *t*-test.

3. Results

3.1. Behavior

There was a positive relationship ($r^2 = 0.084$, $P < 0.01$) between the behavioural response to net restraint (latency to move, number of escape attempts and time spent moving, collapsed into one variable by a PCA) at the initial screening and when re-tested 1.5 months later. Furthermore, fish classified as having a proactive or reactive coping style to net restrain differed significantly in their behavioural response to net restraint when re-tested 1.5 months later ($t = 12$, $P < 0.001$; Fig. 2).

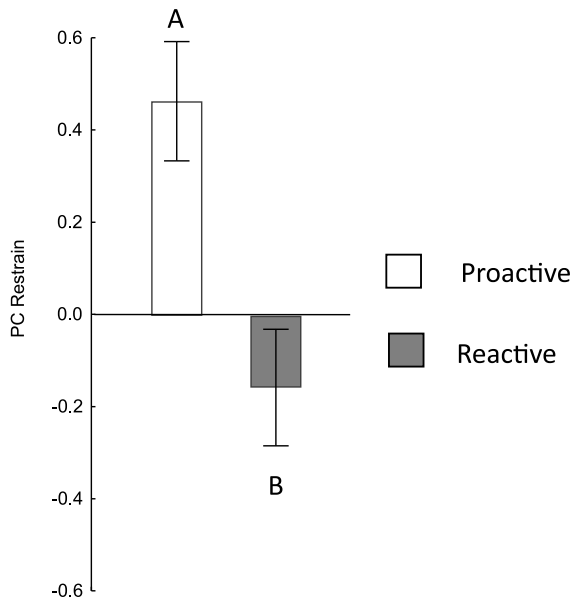


Fig. 2. Principal component score in fish characterised as proactive or reactive stress copers by their behavioural response to net restraint 1.5 month earlier. A higher value corresponds to increased number of escape attempts, a lower latency to escape and showing escape behavior for longer time. Different letters indicate $P < 0.05$.

3.2. Cortisol

Independent of coping style, plasma cortisol showed elevated values 0.5 h post stress compared to baseline values ($P < 0.001$) and plasma concentrations at 2 h post stress ($P < 0.001$; Fig. 3). Furthermore, at 2 h post stress, plasma cortisol levels were not significantly different from baseline values ($P < 0.71$); Fig. 3). However, independent of net restraint there were no significant effects of coping style ($P < 0.13$) on plasma cortisol levels, nor were there any significant interaction effects between coping style and net restraint stress ($P < 0.42$; Fig. 3).

3.3. 5-HT neurochemistry

In the brain stem, the 5-HIAA/5-HT ratios tended to be elevated in reactive fish as compared to proactive fish ($P < 0.051$; Table 2, Fig. 5E). Furthermore, independent of stress coping style, 5-HIAA/5-HT ratios were significantly affected by net restraint ($P < 0.01$; Table 1), resulting in increased values at 0.5 h ($P < 0.001$) and 2 h post stress ($P < 0.001$) compared to baseline 5-HIAA/5-HT ratios in this brain part (Fig. 4F). However, there was no significant difference between 5-HIAA/5-HT ratios at 0.5 and 2 h post stress ($P < 0.97$). In addition, there were no significant interaction effects between stress coping style and restraint stress on 5-HIAA/5-HT ratios in the brain stem. Furthermore, the contrasting stress coping styles were not reflected in significant differences in brain stem 5-HIAA concentrations ($P < 0.26$; Table 2). Still, independent of stress coping style, 5-HIAA levels were

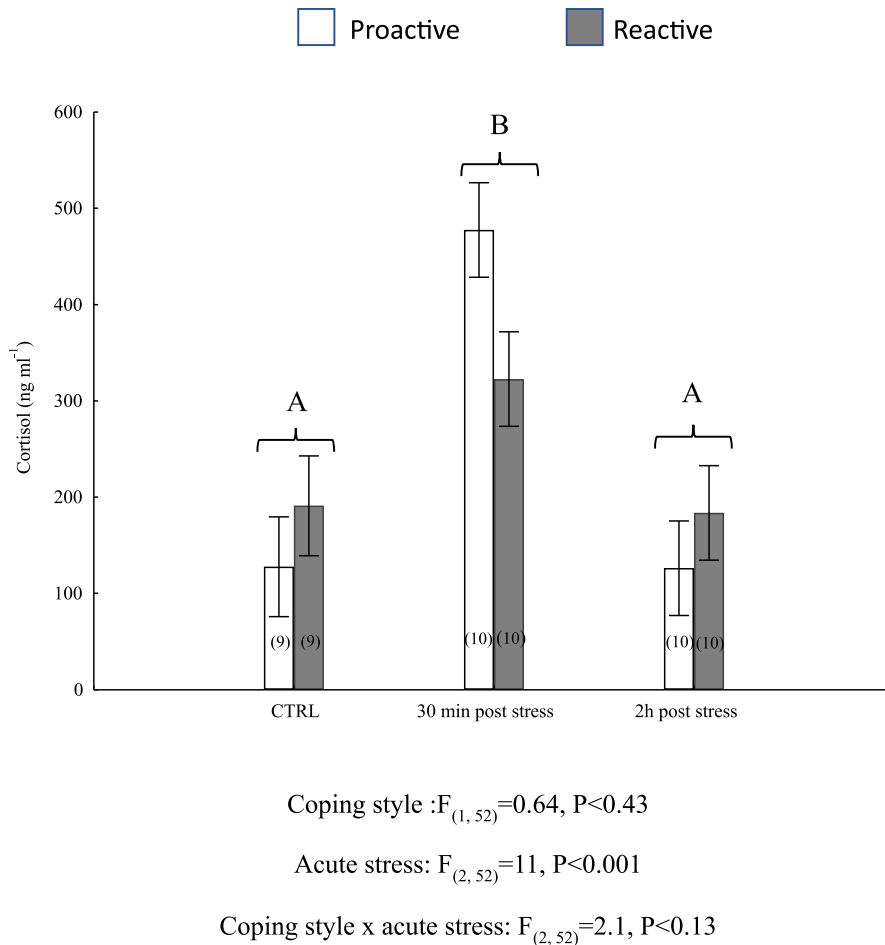


Fig. 3. Plasma cortisol content in Gilthead seabream characterised as proactive or reactive stress copers by their behavioural response to net restraint, before (baseline), 30 and 120 min after being exposed to a second net restraint test. See results for complete statistics from a two-way ANOVAs with stress coping style and sampling time as independent variable. Different letters indicate significant differences ($P < 0.05$), which are independent of behavior response net restraint. Values are mean \pm S.E.M. and number within parentheses are sample sizes.

Table 2
Effects of coping style and acute stress on serotonergic neurochemistry in farmed sea bream. Diverged coping styles were classified by behavioural responses to net restraint and the acute stressor was exposure to five minutes of net restraint.

	ANOVAs		CS x Acute stress	5-HT neurochemistry					
	Coping style			Proactive		Reactive			
	Acute stress			Baseline	0.5 h post stress	2 h post stress	0.5 h poststress	2 h post stress	
Brain stem	5-HT (ng g ⁻¹)	$F_{(1, 47)} = 6.0, P < 0.05$	$F_{(2, 47)} = 1.9, P < 0.16$	230 ± 21 (8)	220 ± 18 (10)	281 ± 22 (7)	208 ± 18 (10)	218 ± 19 (9)	201 ± 19.3 (9)
	5-HIAA (ng g ⁻¹)	$F_{(1, 47)} = 1.3, P < 0.26$	$F_{(2, 47)} = 0.8, P < 0.44$	28 ± 3.4 (8)	37 ± 3.1 (10)	43 ± 3.7 (7)	29 ± 3.1 (10)	37 ± 3.2 (9)	36 ± 3.2 (9)
Telencephalon	5-HIAA/5-HT	$F_{(1, 47)} = 4.0, P < 0.051$	$F_{(2, 49)} = 0.79, P < 0.46$	0.13 ± 0.01 (8)	0.17 ± 0.01 (10)	0.16 ± 0.01 (7)	0.14 ± 0.01 (10)	0.17 ± 0.01 (9)	0.18 ± 0.01 (9)
	5-HT (ng g ⁻¹)	$F_{(1, 47)} = 1.5, P < 0.23$	$F_{(2, 47)} = 1.7, P < 0.18$	311 ± 23 (9)	360 ± 22 (10)	390 ± 22 (10)	329 ± 22 (7)	330 ± 24 (8)	340 ± 22 (9)
	5-HIAA (ng g ⁻¹)	$F_{(1, 47)} = 4.2, P < 0.05$	$F_{(2, 47)} = 1.0, P < 0.36$	31 ± 3.6 (9)	59 ± 3.4 (10)	52 ± 3.4 (10)	40 ± 4.1 (7)	61 ± 3.9 (8)	59 ± 3.6 (9)
	5-HIAA/5-HT	$F_{(1, 47)} = 7.4, P < 0.01$	$F_{(2, 47)} = 0.7, P < 0.50$	0.1 ± 0.01 (9)	0.17 ± 0.01 (10)	0.13 ± 0.01 (10)	0.12 ± 0.01 (7)	0.18 ± 0.02 (8)	0.17 ± (9)
5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin.									

significantly elevated at 0.5 h ($P < 0.05$) and 2 h ($P < 0.01$) post stress compared to baseline values. However, there was no significant interaction of stress coping styles and restraint stress on brain stem 5-HIAA concentrations. In contrast to 5-HIAA, stress coping style was reflected in telencephalic 5-HT concentrations. In this brain part, 5-HT concentrations in reactive fish showed significantly elevated values compared to proactive fish ($P < 0.05$; Table 2).

In telencephalon, there was a significant effect of coping style on 5-HIAA/5-HT ratios. Reactive fish showed higher 5-HIAA/5-HT ratios than proactive individuals ($P < 0.01$) (Table 2, Fig. 5E). Moreover, independent of stress coping style, net restraint stress resulted in elevated telencephalic 5-HIAA/5-HT ratios at 0.5 h ($P < 0.001$) and 2 h ($P < 0.01$) post stress compared to baseline values (Table 1). Furthermore, telencephalic 5-HIAA/5-HT ratios at 0.5 h post stress were significantly higher than those at 2 h post stress ($P < 0.05$; Fig. 4F) but there was no significant interaction between coping styles and net restraint stress in this brain part ($P < 0.46$; Table 2). Net restraint also affected telencephalic 5-HIAA concentrations independently of coping styles (Table 2). 5-HIAA levels were significantly higher at 0.5 h post stress compared to 2 h post stress ($P < 0.01$) and baseline values ($P < 0.005$; Fig. 5D). Moreover, 5-HIAA levels were not significantly different from baseline values at 2 h post stress ($P = 0.32$). Independent of net restraint, there was a trend towards elevated 5-HIAA concentrations in the telencephalon of proactive individuals ($P = 0.062$) but there was no significant interaction between coping style and net restraint ($P = 0.31$; Table 2). In addition, neither net restraint ($P = 0.37$) nor coping style ($P = 0.56$) had any significant effects on telencephalic 5-HT concentrations and neither were there any interaction effect ($P = 0.26$; Table 2).

4. Discussion

In this study we show that consistent differences between a reactive and proactive response to net restraint is reflected in 5-HT concentrations in brainstem and in telencephalic 5-HT turnover (5-HIAA/5-HT ratio). Furthermore, independent of the contrasting behavioural responses, net restraint resulted in elevated plasma cortisol 0.5 h post restraint. In addition, restraint stress resulted in increased 5-HIAA concentrations and 5-HT turnover in brain stem and telencephalon at 0.5 and 2 h post restraint.

Generally, behavioural responses to net restraint have been shown to be associated with other behavioural and/or physiological responses to challenges in teleost fish. In a previous study [28] it was demonstrated that more escape attempts during net restraint in sea bream was associated with other proactive behavioural traits, including shorter time to leave a familiar area [29] and avoidance to hypoxia [30]. The fact that these behavioural associations were not reflected in post stress plasma cortisol levels made the authors suggest that behavior characteristics of the proactive-reactive axis was independent of HPI axis reactivity in seabream. In accordance with this, Ibarra-Zatarain [31] reported that there were no differences in cortisol response between Senegalese sole (*Solea senegalensis*) with high respective low number of escape attempts during net restraint. However, contrasting results have been reported. For example, Magnhagen et al. [32] demonstrated a negative relationship between the behavioural response to net restraint and the plasma cortisol response to 0.5 h confinement stress in Arctic charr (*Salvelinus alpinus*). Likewise, Ferrari et al. [33] reported a negative relationship between struggling during net restraint and plasma cortisol levels 30 min post net restraint in seabass (*Dicentrarchus labrax*). Our study demonstrates that individuals showing consistent contrasting responses to net restraint do not differ in post stress plasma cortisol. This is in accordance with the studies performed by Casteria et al. [28] and Ibarra-Zatarain et al. [31] and supports the idea that stress coping styles can be independent of HPI axis reactivity. It has been suggested that different neuroendocrine characteristics are mainly a consequence rather than the cause of the behavioral differentiation, and that

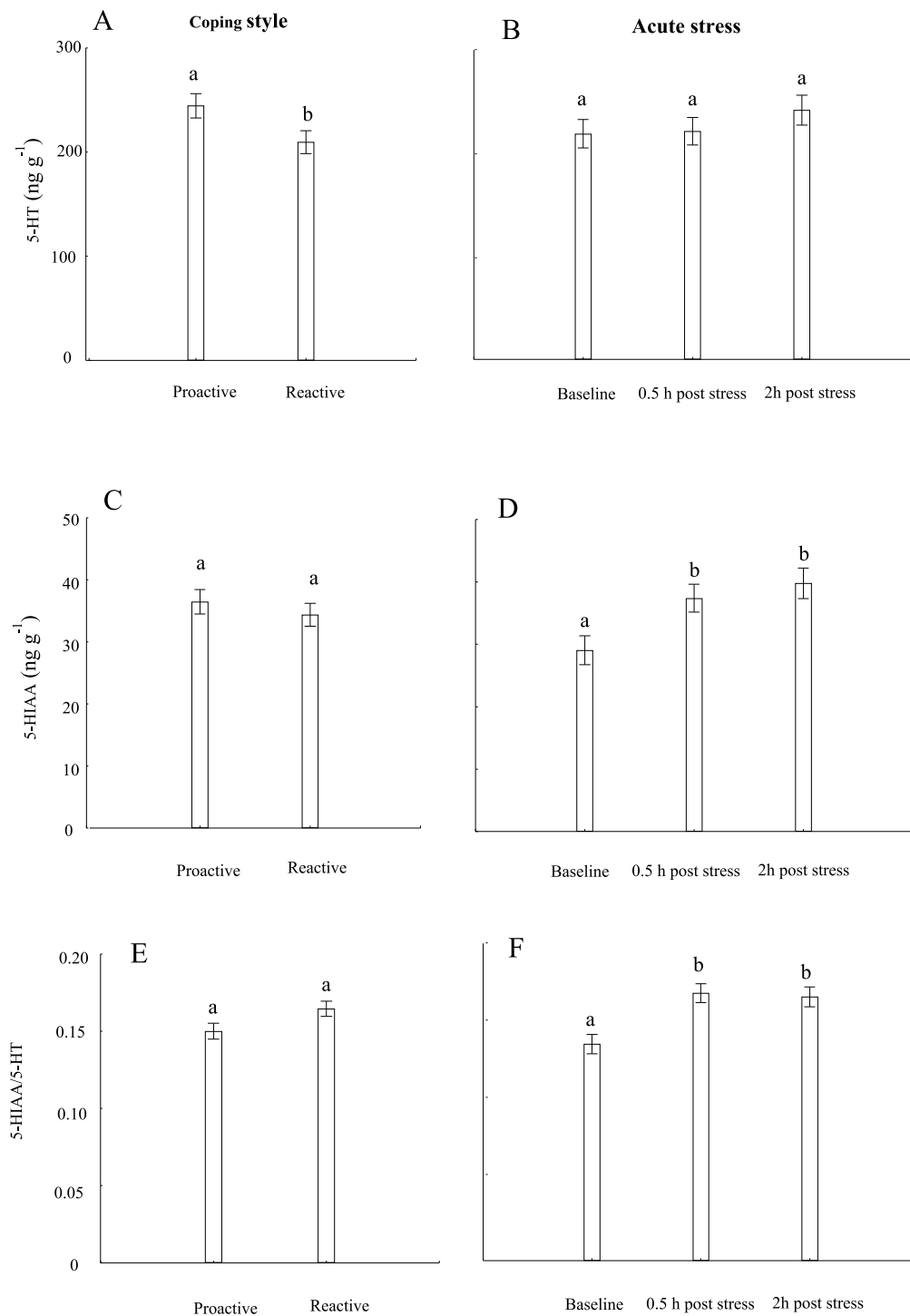


Fig. 4. Serotonergic neurochemistry in telencephalon of Gilthead seabream previously characterised as proactive or reactive stress copers by their behavioural response to net restraint, before (baseline), 30 and 120 min after being exposed to a second net restraint test. Values are from a two-way ANOVA with type of behavioural response to net restraint and effects of acute net restraint as independent variables. See results and [Table 1](#) for complete statistics from the two-way ANOVA. Different letters indicate significant differences ($P < 0.05$). Values are mean \pm S.E.M.

correlations with coping style should be considered as the consequence of the differential appraisal of the environmental challenge [2]. In the light of this, further studies are needed to clarify to what extent differences in appraisal underlies the inconsistency in the relationship between HPI axis reactivity and behavioral response to net restraint in teleost fishes. Furthermore, it is important to keep in mind that the stress response is a dynamic process, and both dynamics and magnitude of the stress response might be reflected in contrasting stress coping

styles [26]. Thus, it cannot be excluded that differences in the dynamics of the stress response concealed potential divergences in HPI axis activity of proactive and reactive fish in the present study.

Our study shows that seabream characterized by a proactive response to net restraint show lower 5-HIAA concentrations and 5-HIAA/5-HT ratios along with higher 5-HT levels in the telencephalon as compared to fish characterized as reactive. Since 5-HIAA concentrations and 5-HT turnover rate is related to 5-HT release and metabolism

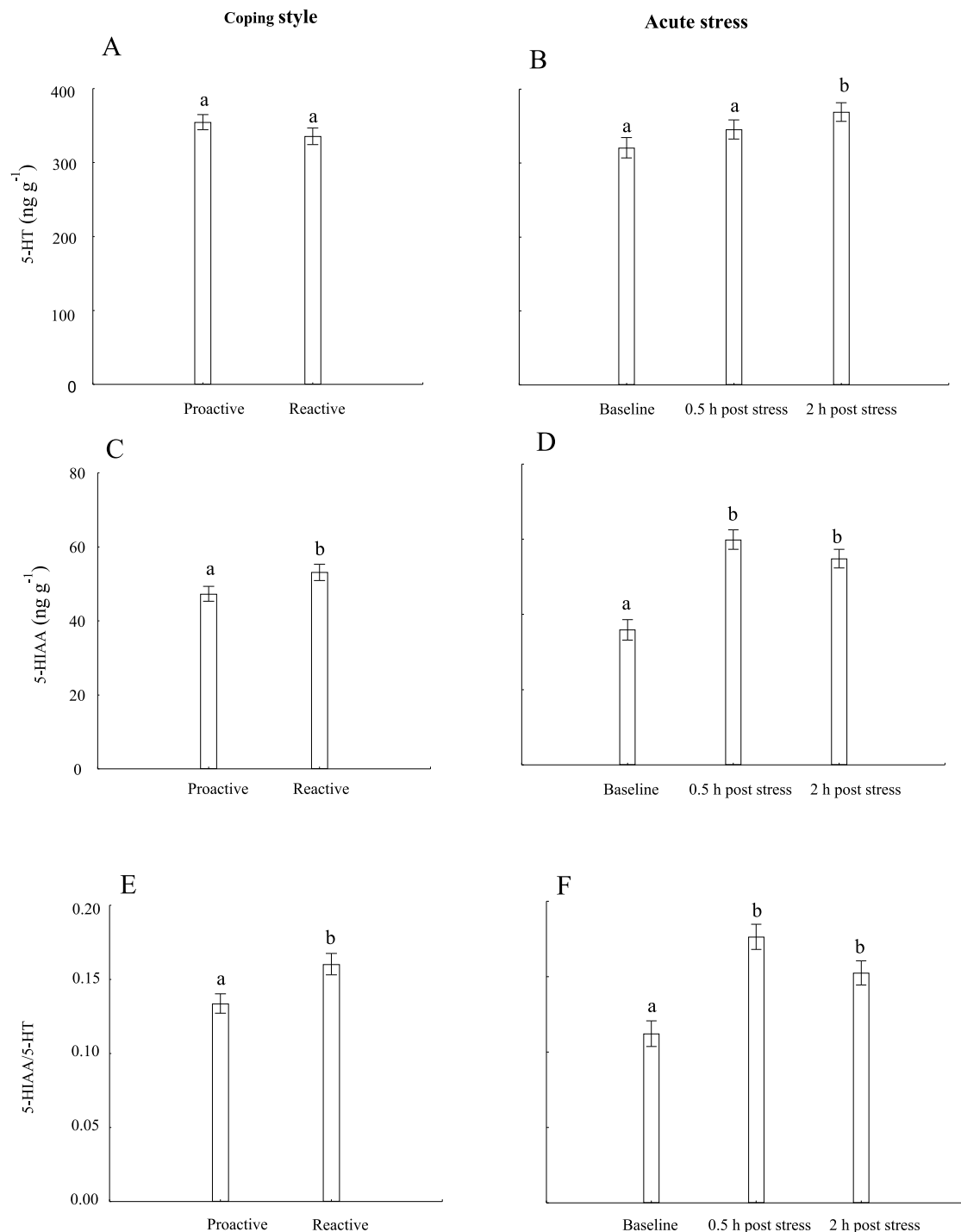


Fig. 5. Serotonergic neurochemistry in brain stem of Gilthead seabream previously characterised as having an active passive or behavioural response to net restraint, before (baseline), 30 and 120 min after being exposed to a second net restraint test. Values are from a two-way ANOVA with type of behavioural response to net restraint and effects of acute net restrain as independent variables. See results and [Table 1](#) for complete statistics from the two-way ANOVA. Different letters indicate significant differences ($P < 0.05$). Values are mean \pm S.E.M.

[34], our results suggests that a proactive stress coping style is associated with a lower 5-HT release and/or metabolism in the telencephalon. Furthermore, 5-HT neurons in the raphe nuclei of the brainstem show an extremely divergent projection pattern, including projections to forebrain areas [35]. Thus, a higher brain stem 5-HT concentration in proactive fish showing an active response to net restraint could be an effect of generally lower 5-HT release/metabolism in telencephalon and/or a higher 5-HT production in brain stem. Compared to the HR-LR trout model, the current results show some

similarities. As in our study, Schjolden et al. [26] reported differences in 5-HT turnover rate but could not detect any differences in post stress telencephalic 5-HT turnover rate between the HR (reactive) and LR (proactive) line. However, in contrast to our study LR trout displayed higher baseline telencephalic 5-HT turnover rate as compared to HR trout. Furthermore, post stress brainstem 5-HT turnover rate and 5-HIAA concentrations were elevated in fish of the HR line compared to those of the LR line. Such stress induced differences in brain stem 5-HT metabolism were not seen in our study. It has been suggested that stress

reactivity and stress coping style represent different independent behavioural axes, where stress reactivity refers to the magnitude of the response and stress coping style refers to the quality or type of response [36]. Considering that it is currently unknown to what extent 5-HT signaling is reflected in the stress coping or stress responsiveness dimensions [3], it is important to note that the HR-LR trout lines are selected on post stress plasma cortisol levels while the current study is performed on fish characterized as proactive or reactive by their behavioural response to net restraint. Furthermore, aggression is a behavioural trait which has been suggested to reflect individual variation in the more general active-passive continuum [37]. Following this idea, Koolhaas et al. [2], suggested that differences in 5-HT control of the brain regions associated with aggression, such as the hypothalamic attack area and the prefrontal cortex, could be associated with contrasts in stress coping style rather than with the emotional axis. Less is known about the brain circuits involved in aggression in teleost fish [38]. Thus, further studies of 5-HT brain neurochemistry, especially focusing on potential regions involved in aggressive behavior, might disclose the involvement of this neurotransmitter in the dissociation between the stress coping style and stress responsiveness axis in teleost fish.

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Supplementary materials

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