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Tactile stimulation enrichment affects behavioural and physiological responses of a marine commercial fish species, the gilthead seabream *Sparus aurata*

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ABSTRACT

Fish welfare is gaining increasing attention in both public awareness and research, reflecting a growing recognition and appreciation of its ethical importance in aquaculture. As in nature, reared fish exhibit a range of behaviours and physiological responses suggesting their capacity to experience pain and distress when exposed to deleterious stimuli, however there is a pressing need to aim at the positive aspects of sentience. This study investigated physical and tactile contact as a potential enrichment method for reducing stress and improving welfare in the gilthead seabream *Sparus aurata* (Linnaeus, 1758). To provide tactile contact, an apparatus made of plastic rods and silicone bristles was used as treatment, while a control setup included only the plastic rods, without the silicone bristles. The experiment lasted 21 days, with fish housed in groups of four per tank. Behavioural assessments were conducted through daily 30-min video recordings, quantifying fish crossing through the bristles, and aggressive displays and attacks. Additionally, half of the fish underwent a confinement stress test on the 22nd day, with cortisol levels and brain monoamines measured to evaluate metabolic stress responses and brain signalling. Individuals in the bristle enrichment group were less inclined to display aggression and showed lower cortisol response after acute stress. Regarding brain monoaminergic activity, differences occurred mostly at two main regional sections: midbrain (central brain section) and hindbrain, both involved in seabream response to acute stress and in connection with bristle enrichment. This is the first demonstration that exposure to tactile stimulation during a limited amount of time is enough to produce behavioural change, mitigate stress responses and modify brain monoaminergic signalling in this important commercial species.

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1. Introduction

Aquaculture currently supplies more than half of the fish consumed worldwide and depends critically on maintaining high standards of animal health and welfare to ensure productivity and sustainability (FAO, 2022). Several stress factors affect reared fish, which can impact key physiological processes, including osmoregulation, immune function, energy metabolism, and behaviour, often resulting in reduced overall performance and welfare in fish (Huntingford et al., 2006; Parma et al., 2019; Van De Vis et al., 2020). By advancing research in fish welfare, it will be possible to develop evidence-based practices that improve welfare and align with ethical standards and consumer expectations. In this context, fish welfare is receiving increasing attention in both research and public awareness, reflecting a growing recognition of its ethical, ecological, and economic importance (Planellas et al., 2026). Studies show that fish exhibit a range of behaviours, physiological responses, and stress indicators, suggesting their capacity to experience pain and distress (Brown, 2015; Saraiva and Arechavala-Lopez, 2019). However, most of the research on these issues in fish has been focused on negative aspects, such as pain, stress, distress, and suffering and their consequences; while there is a pressing need to aim at the positive aspects of sentience, which are relevant to fields from evolutionary biology to animal welfare (Balcombe, 2009; Fife-Cook and Franks, 2019). Indeed, positive welfare has emerged as an important concept in animal welfare science. Overall, it recognizes the importance of providing animals with the opportunity for rewarding experiences and fulfilled lives, beyond the alleviation of pain and suffering (Rault et al., 2023). This scientific approach aligns with common societal views on animal welfare, which states that animals should be provided with opportunities for rewarding experiences, building upon the recognition that positive emotions are not exclusive to humans and may bring an adaptive advantage to animals' fitness (Fraser and Duncan, 1998). For instance, in fish, such rewarding experiences may include the opportunity to express exploratory behaviour, to engage in feeding activities or to socialize with other conspecifics, all of which may increase motivation, interest and comfort (Arechavala-Lopez et al., 2021).

In the wild, fish are exposed to highly complex and diverse environments, resulting in a wide array of sensory stimuli. In contrast, captive settings such as aquaculture, often lack this diversity of environmental cues. An effective way to enhance fish welfare in captive or laboratory conditions and to promote positive and rewarding experiences is through the implementation of environmental enrichment strategies (Näslund and Johnsson, 2016) which may include: physical enrichment (such as providing structures for shelter, substrates or a combination of both), sensorial enrichment (visual, auditory, chemical or tactile), occupational enrichment, social and even dietary enrichment (for review see Arechavala-Lopez et al., 2021). Naturally, the benefit of implementing any sort of enrichment will depend on the biology of the species in question and on the specific characteristics of the enrichment strategy (Jones et al., 2021). Thus, it is vital to test and adjust it beforehand, focusing on the behavioural and physiological response of the species.

Interest in the rewarding effects of physical or tactile stimulation started from observations of mutualistic cleaning behaviour in fish, where the act of being cleaned is thought to provide a putative physiological reward (Soares, 2017). In interspecific fish-fish interactions, the host, also known as the client fish, regularly seek other smaller, specialized fish (cleaners) to have their body surface, gill chambers and mouth inspected for ectoparasites, fungal growths, and dead and diseased tissues (Caves, 2021). During these interactions, cleaners often straddle the back of their clients and provide a “massage” with their pelvic and pectoral fins - a behaviour termed tactile stimulation (Bshary and Würth, 2001; Grutter, 2004). A pivotal study then found that clients not only benefit from parasite reduction but also have their cortisol levels reduced from physical contact alone (Soares et al., 2011). Additionally, similar studies have demonstrated that physical contact reduces anxiety in zebrafish (Schirmer et al., 2013) and aggression in male Nile tilapia kept in isolation (Bolognesi et al., 2019). When testing longer-term effects (e.g., 21 days), Gauy et al. (2022) showed that it reduced aggression more markedly in male Nile tilapia kept in groups, increasing growth and improving other productive parameters. For this species, physical contact reduces the negative effects of high stocking density improving feeding efficiency and body condition factors (Gauy et al., 2023). Physical stimulation may, therefore, be considered a type of sensorial enrichment that may be a promising tool to promote fish welfare.

But beyond cortisol, other physiological indicators (such as brain monoamines) can also be informative, which together with behavioural metrics may become a powerful and reliable tool for assessing fish welfare (Brandão et al., 2021). Monoaminergic systems in the brain of vertebrates are generally organized as groups of cell bodies (nuclei) localized in specific brain areas that send axonal projections across the brain, forming networks with modulatory actions on different processes (Lillesaar, 2011; Yamamoto and Vernier, 2011; Winberg, 2023). Alterations in monoamine levels and/or monoaminergic activity due to different process or events, such as acute stress, often occurs in a generalized way, with parallel changes across different brain areas (Gesto et al., 2013; López-Patiño et al., 2021). However, alterations are also often observed in specific brain areas (Winberg, 2023), pointing to more localized changes that could be related to the modulation of specific processes. For instance, DA is involved in decision-making, learning and reward mechanisms (Messias et al., 2016a, 2016b; Schultz, 2002, 2005; Soares, 2017) and 5-HT in helping animals to cope with stress (Dahlbom et al., 2011; Teles et al., 2013), and both serotonergic and dopaminergic systems have been linked to stress in fish as in other vertebrates (Winberg, 2023).

In this study we tested the implementation of body sensory enrichment, a tactile stimulation apparatus, in a relevant commercial species: the gilthead seabream *Sparus aurata* (Linnaeus, 1758). *S. aurata* is one of the most widely produced aquaculture species in the Mediterranean region (FAO, 2022; Mhalhel et al., 2023) but increased production pressure has intensified several stressors, with high rearing densities being a well-documented source of chronic stress (Parma et al., 2019). These conditions can impact key physiological processes, including osmoregulation, immune function, energy metabolism, and behaviour, often resulting in reduced overall performance and welfare in fish (Parma et al., 2019). While tactile stimulation has been extensively investigated in species that naturally engage in cleaning interactions, its relevance may extend beyond these contexts. For *S. aurata*, which occupies diverse coastal habitats and exhibits both social and benthic behaviours, tactile cues may arise through interactions with conspecifics, the substrate, and the physical environment. Thus, the present study does not aim to reproduce a specific natural tactile interaction but rather evaluates whether controlled tactile stimulation can function as a sensory enrichment approach under aquaculture conditions. We hypothesize that *S. aurata* will be able to interact with the tactile stimulation apparatus over time, and that such interactions will (i) reduce aggressive behaviour, (ii) attenuate cortisol responses to acute stress, and (iii) modulate monoaminergic activity in brain regional sections associated with stress (confinement) and reward processing (tactile stimulation), showing generalized but also section-specific patterns.

2. Methods

2.1. Study species and housing conditions

S. aurata individuals were originated from the broodstock of the Aquaculture Research Station of the Portuguese Institute for the Sea and Atmosphere (IPMA, I.P.). Individuals were immature juveniles with standard length and weight, respectively (mean ± S.E.): 10.24 ± 0.08 cm, 34.22 ± 1.04 g. Fish were acclimated for a minimum of 20 days in a glass tank (118 × 45 × 55 cm; ca. 270 L). This tank, along with 12

smaller tanks (40 × 50 × 40 cm; ca. 80 L) was part of a seawater recirculating system equipped with a biofilter, air supply, and a protein skimmer. Each tank was equipped with aeration system. Water quality parameters were monitored with commercial kits (Tropic Marin®): Ammonia and Nitrite/Nitrate (below 0.2 ppm), and pH (7.7); salinity was kept at 30.2 ‰ and temperature at 21 °C. The light regime was 12 L:12 D (6:00 a.m. to 6:00 p.m.). Fish were fed to apparent satiety with a commercial feed formulated for seabream, twice daily (8:00 a.m. and 4:00 p.m.)

2.2. Experimental setup and behavioural assessment

To simulate tactile stimulation in the laboratory, we developed an apparatus consisting of a polyvinyl chloride (PVC) tube frame fitted with vertical plastic sticks lined with soft silicone bristles on their sides (Fig. 1A). This design has shown to successfully make contact with the fish as they pass through without removing mucus or causing injury (Bolognesi et al., 2019; Gauy et al., 2022, 2023). We used a similar apparatus containing sticks without silicone bristles as a control (Fig. 1B). A total of 64 *S. aurata* individuals were allocated into 16 groups of four fish each (Fig. 1C), in two experimental rounds. On the following day, groups were randomly assigned to either the tactile stimulation treatment (apparatus with bristles: $n = 8$ groups, 32 individuals) or the control (apparatus without bristles; n = 8 groups, 32 individuals), following the protocol by Gauy et al. (2022), with fish size balanced across groups and treatments. Two Logitech® C615 cameras were mounted above each aquarium to record the fish for 30 min/day (always between 12:00 p.m. and 2:00 p.m.), six days a week in a total of 18 days (Fig. 1D) to quantify: a) the number of crossings through the apparatus and b) the number of aggressive interactions. Aggressive interactions were labelled as attacks (physical contact aggressive behaviours such as chases or fights involving bites) or displays (non-contact aggressive behaviours such as chases without physical contact). Video recordings were analysed using the Behavioural Observation Research Interactive Software - BORIS (Friard and Gamba, 2016).

All sampling took place on the morning of the 22nd day (Fig. 1D). Half of the treated and control individuals were subject to a confinement stress test, a standardized method for measuring acute stress responses in fish (Soares et al., 2011, see Fig. 1E). The confinement test entailed transferring each fish to a small aquarium (see Fig. 1E for size) with a reduced amount of water (water depth of 10 cm) for 30 min. All individuals from both treatments were euthanized, regardless of whether they were subjected to the stress test, with an overdose of phenoxyethanol. After being euthanized, the fish were weighed, and the heads and gills (only left gill) were separated from the body, with heads and gills stored at −80 °C and bodies at −20 °C.

2.3. Cortisol and brain monoamine analyses

Cortisol was assayed from gill tissue according to previously described protocol (Gesto et al., 2015). We decided to use of gill cortisol over plasma cortisol due to size of the fish. Since getting blood from smaller fish can be difficult and takes longer avoiding the potential influence of sampling (sampling time and sampling order) on cortisol levels. Briefly, gill samples were homogenized in 500 μL of phosphate buffer saline (pH 7.4) using a pestle for Eppendorf-like tubes, followed by application of ultrasounds (Sonopuls HD3100, Bandelin, Germany). Homogenates were centrifuged (14,000 xg, 10 min), and supernatants were assayed using a commercial cortisol ELISA kit (Tecan/IBL RE52611). Soluble protein content of the supernatants was analysed with the bicinchoninic acid method (Smith, 1985) using a commercial kit (Ref 23,225, Thermo Scientific, Rockford, IL, USA). Cortisol levels were normalized by the protein content of the gill homogenates.

For the collection of brain samples and brain section separation, fish heads were removed from the freezer and left for 4 min at room temperature. The roof of the skull was carefully removed with a scalpel to expose the brain dorsally. The telencephalon (referred here as forebrain) was dissected out first by performing vertical cuts with a scalpel to separate the telencephalic lobes from anterior (olfactory bulb) and posterior (optic tectum and rest of the brain) structures. After that, another vertical cut was performed at the line separating the optic tectum from the cerebellum, dividing the brain into a central section (referred as midbrain) and a posterior section (referred as hindbrain). The midbrain contained the diencephalon (including hypothalamus) and optic tectum (the tegmentum) areas (Calvo and Schluessel, 2021). The posterior section corresponded to hindbrain (rhombencephalon)

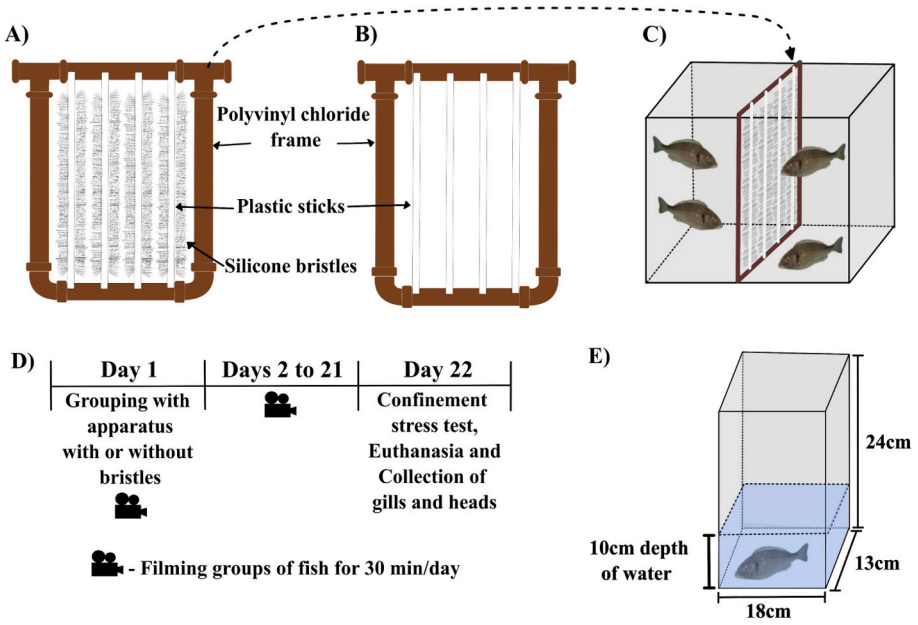

Fig. 1. Tactile stimulation apparatus and experimental design. The apparatus for tactile stimulation was made of a rectangular PVC structure with silicone bristles attached to plastic sticks (A), the controlled apparatus without bristles (B), and an apparatus placed in the centre of the aquarium (C). Experimental schedule (D): fish were grouped on day 1 and allocated either for tanks with silicone bristle apparatus or control. Fish were then filmed (30 min/day) from day 1 to day 21. On the 22nd day, 2 individuals of each group (tank) went through a confinement stress test (see E) and all were euthanised (see methods).

structures including the cerebellum dorsally, and the myelencephalon (medulla oblongata) ventrally (Calvo and Schluessel, 2021). The dissection took less than 2 min and brain tissue was still semi frozen when finished. While we consider the precision of the brain dissection in 3 large regional sections to be very good, we do acknowledge that it may not be perfect; however, results during preliminary tests confirmed that regions were accurately separated. Integrity was kept by performing the dissection while the heads and brains were still not completely thawed, brain tissue was collected as semi-frozen tissue blocks that were quickly allocated on ice before quick transferring the tubes to —80C freezer. The duration of the dissection was lower than 2 min. The monoamines DA (dopamine), NA (noradrenaline), DOPAC (3,4-dihydroxyphenylacetic acid), 5-HIAA (5-hydroxyindoleacetic acid) and 5-HT (serotonin) were assessed in the three parts of the brain: forebrain, midbrain and hindbrain. All brain tissues were weighed and homogenized in 400 μL of perchloric acid (PCA) solution (0.4 M PCA and 0.1 mM EDTA) using an ultrasonicator (Sonopuls HD3100, Bandelin, Germany). After centrifugation, (14,000 xg, 4 °C, 10 min), supernatants dilutions were assayed by high-performance liquid chromatography (HPLC) analysis. Samples were analysed by HPLC with electrochemical detection (HPLC-EC). Chromatographic separation was done using a Supelcosil LC-18-dB column (15 cm × 4.6 mm, 5 μm) (Supelco, Bellefonte, PA, USA). The HPLC mobile phase consisted of 73.9 mM NAH2PO4, 0.1 mM Na_2EDTA and 0.58 mM sodium 1-octanesulfonate in deionized water with 15.3% (*v*/v) methanol, pH = 3.0 (Gesto et al., 2013). The detection system included a 5011 A analytical cell with oxidation potentials set at +200 mV (first electrode) and -350 mV (second electrode). A conditioning cell (5020) at +300 mV was used before the analytical cell. DOPAC/DA and 5-HIAA/5-HT mass ratios were calculated and used as proxy of dopaminergic and serotonergic signalling in the assessed brain tissues (Winberg and Nilsson, 1993).

2.4. Statistical analysis

Data was checked for homogeneity, normality, collinearity, and possible outliers (Zuur et al., 2010). For fish behaviour, because animals were not marked, each tank were deemed as an experimental unit and the variables considered were: crossings, displays and attacks were analysed between treatments (with tactile stimulation vs without tactile stimulation), considering the treatment as a categorical factor and days (time) of observation as a repeated measure (Mixed Model ANOVA). Regarding fish cortisol and monoaminergic levels, analyses were made to an individual level, with two-way factorial ANOVAs, with treatment (with tactile stimulation vs without tactile stimulation) and stress confinement test (yes, no) as factors. The initial model included tank id as random factor however, because retaining the factor did not improve model fit or affected inference of the fixed effects, it was removed from the final model. Therefore, the simpler model is presented. Planned comparisons were used to contrast between and within treatments. Statistical significance was set at $p \leq 0.05$. Data were analysed in Statistica (StatSoft) package.

2.5. Ethical note

Animal procedures used in this study were approved by the ethical committee of CIBIO-BIOPOLIS, reference 2023–01. The facilities at Aquário Vasco da Gama where the study was conducted are licenced by DGAV – Direção Geral da Alimentação e Veterinária. All experiments were performed in accordance with the relevant guidelines and regulations of the ethical committees.

3. Results

3.1. Fish behaviour

Groups of *S. aurata* were observed to cross the bristle- equipped apparatus (mean number of apparatus crossings ± SD: 24.1 ± 20.5, per 30 min daily). Indeed, groups assigned to the control apparatus (without bristles) crossed significantly more than those with bristle treatment (bristle treatment, Mixed Model ANOVA: $F_{(1,14)} = 30.49$, $p < 0.001$,

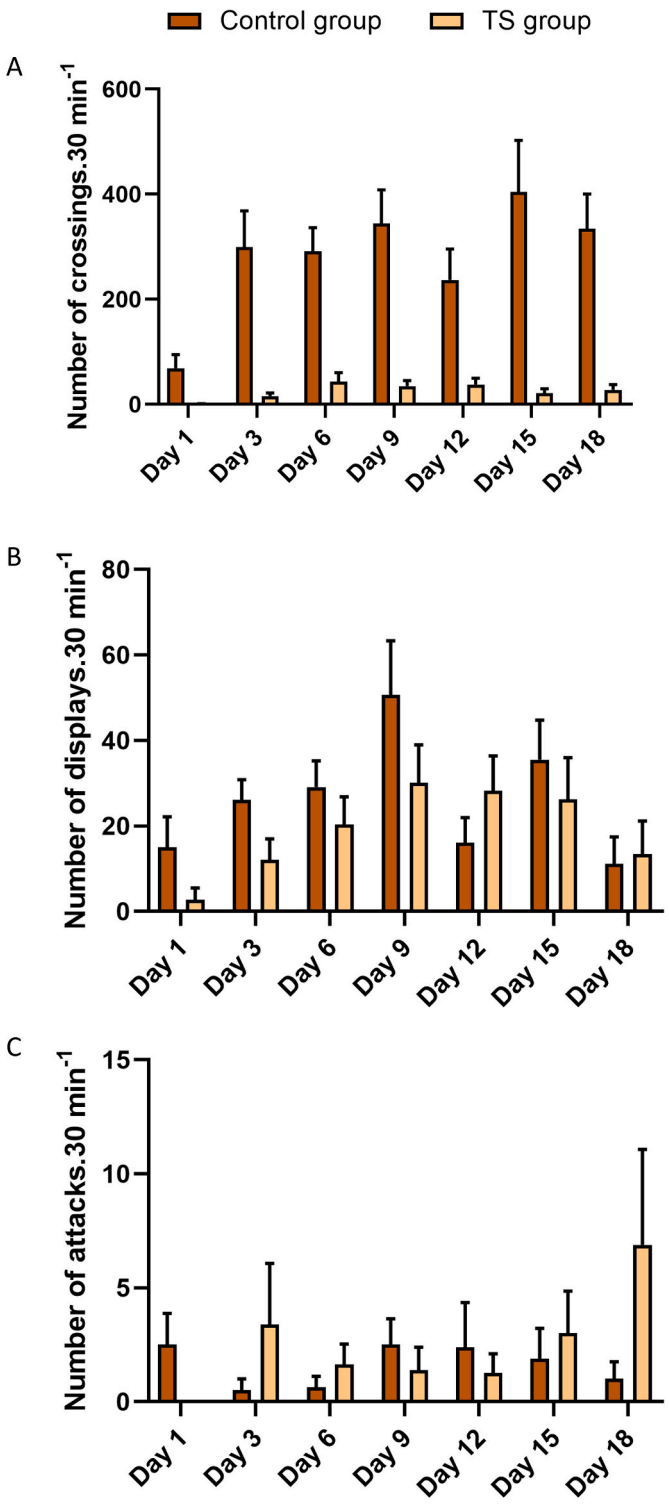

Fig. 2. Behavioural response to the tactile stimulation apparatus or control. *Sparus aurata* number of crossings through the bristle apparatus and control (A), number of displays (B) and number of attacks (C) for 18 days of fish grouping for control group (orange) and tactile stimulation group (yellow). Data are Mean ± S. E. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 2A). We found significant differences across observation days (time, Mixed Model ANOVA: $F_{(1,6)} = 6.62$, $p < 0.001$), but no interaction between time and treatment (interaction, Mixed Model ANOVA: $F_{(6,89)} = 2.16$, $p = 0.054$, Fig. 2A). Displays in *S. aurata* also differ, with fish in the control group showing more aggressive displays than those in the bristle treatment (bristle treatment, Mixed Model ANOVA: $F_{(1,14)} = 5.51$, $p = 0.034$, Fig. 2B), with significant differences across days (time, Mixed Model ANOVA, $F_{(1,6)} = 2.61$, $p = 0.022$) and no interaction between time and treatment (interaction, Mixed Model ANOVA: $F_{(6,89)} = 0.62$, $p = 0.71$). Regarding overt aggressions (attacks), no difference was found between treatment (bristle treatment, Mixed Model ANOVA: $F_{(1,14)} = 0.01$, $p = 0.91$, Fig. 2C), or observation days (time, Mixed Model ANOVA: $F_{(1,6)} = 1.42$, $p = 0.21$), but we found an interaction between time and treatment (interaction, Mixed Model ANOVA: $F_{(6,89)} = 3.48$, $p = 0.004$).

3.2. Fish cortisol levels

Confinement had a significant effect on the increase of cortisol levels in *S. aurata* (confinement, Two-way ANOVA: $F_{(1,59)} = 58.02$, $p < 0.001$), regardless of bristle treatment (bristle treatment, Two-way ANOVA: $F_{(1,59)} = 1.90$, $p = 0.17$) and with no interaction between bristle treatment and confinement (interaction, Two-way ANOVA: $F_{(1,59)} = 2.41$, $p = 0.12$). Contrasts by planned comparisons revealed that previous exposure to bristles reduced cortisol levels amongst those that experienced confinement test but not necessarily amongst those that were unstressed (Fig. 3, see Table 2).

3.3. Fish brain monoamine levels

3.3.1. Brain NA and DA metabolite levels

Confinement also influenced significantly the ratios of DOPAC/DA at the midbrain and hindbrain of *S. aurata*, but not at the forebrain (confinement, Two-way ANOVAs, (forebrain): $F_{(1,59)} = 3.39$, $p = 0.07$; (midbrain) $F_{(1,59)} = 4.63$, $p = 0.03$ and (hindbrain): $F_{(1,59)} = 5.82$, $p = 0.02$), regardless of bristle treatment (bristle treatment, Two-way ANOVAs, (forebrain): $F_{(1,59)} = 1.48$, $p = 0.23$; midbrain – $F_{(1,59)} = 0.29$, $p = 0.59$ and hindbrain $F_{(1,59)} = 1.21$, $p = 0.27$) and with no interaction between bristle treatment and confinement (interaction, Two-way ANOVAs: forebrain - $F_{(1,59)} = 0.34$, $p = 0.56$; midbrain – $F_{(1,59)} = 0.07$, $p = 0.80$ and hindbrain – $F_{(1,59)} = 0.01$, $p = 0.92$). DOPAC levels at the forebrain, midbrain and hindbrain were also responsive to confinement (confinement, Two-way ANOVAs: forebrain – $F_{(1,59)} = 6.29$, $p = 0.01$; midbrain – $F_{(1,59)} = 10.96$, $p = 0.001$ and hindbrain - $F_{(1,59)} = 4.89$, $p = 0.03$), regardless of bristle treatment (bristle treatment, Two-way ANOVAs: forebrain – $F_{(1,59)} = 3.23$, $p = 0.07$; midbrain – $F_{(1,59)} = 0.63$, $p = 0.43$ and hindbrain – $F_{(1,59)} = 0.07$, $p = 0.79$) and with no interaction between bristle treatment and confinement (interaction, Two-way ANOVAs, (forebrain): $F_{(1,59)} = 0.005$, $p = 0.94$; (midbrain): $F_{(1,59)} = 0.34$, $p = 0.56$ and (hindbrain): $F_{(1,59)} = 0.09$, $p = 0.76$). No significant results were found for NA levels across brain sections (see Table 1). Contrasts by planned comparisons revealed that amongst those exposed to bristles, individuals following confinement had significantly higher DOPAC levels at the midbrain (see Table 2 for complete analyses).

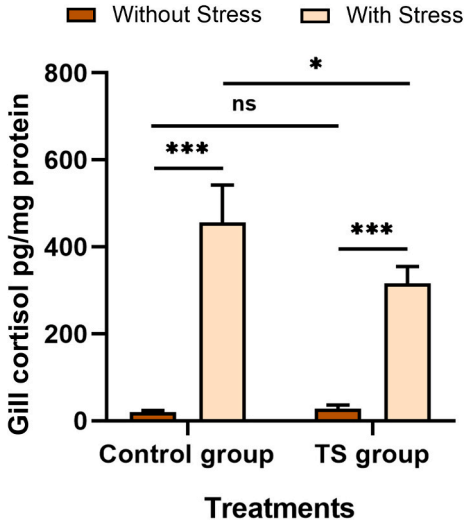

Fig. 3. Cortisol response to the tactile stimulation apparatus or control. Gill cortisol levels of *Sparus aurata* individuals that were either assigned to treatment (tactile stimulation apparatus) or control. Within each group, individuals were either exposed to a confinement stress test (confined group — light bars) or remained unconfined (unconfined group — dark bars). Asterisk indicates significant differences within treatments after planned comparisons (* $p < 0.05$ and ** $p < 0.01$). See complete results in Table 2. Data are shown as mean ± SE.

3.3.2. Brain 5-HT metabolite levels

As for the 5-HIAA/5-HT ratios in *S. aurata*, no significant effects were found following confinement, treatment and in the interaction between bristle treatment and confinement (all $p > 0.05$, see Table 1) in the forebrain and midbrain, except for the hindbrain where bristle treatment was found to significantly influence their levels (bristle treatment, Two-way ANOVA (hindbrain): $F_{(1,59)} = 4.24$, $p = 0.04$), regardless of confinement stress test (confinement, Two-way ANOVA (hindbrain): $F_{(1,59)} = 0.02$, $p = 0.87$) and with no interaction between the variables (interaction Two-way ANOVA (hindbrain): $F_{(1,59)} = 0.001$, $p = 0.97$). 5-HIAA levels at the hindbrain were also found to differ with bristle treatment (bristle treatment, Two-way ANOVA (hindbrain): $F_{(1,59)} = 4.70$, $p = 0.03$), regardless of confinement stress test (confinement, Two-way ANOVA (hindbrain): $F_{(1,59)} = 0.95$, $p = 0.33$) and with no interaction between the variables (interaction, Two-way ANOVA (hindbrain): $F_{(1,59)} = 0.50$, $p = 0.48$). Contrasts by planned comparisons showed that previous exposure to bristles increased 5-HIAA levels at the hindbrain, amongst those that experienced confinement but not necessarily amongst those that were unstressed (see Table 2 for complete analyses). All remaining monoamines analyses and all contrasts by planned comparisons are shown in Tables 1 and 2.

4. Discussion

Groups of *S. aurata* were crossing the bristle apparatus during the 21 days of exposure, which means they were receiving sensorial contact. Because groups of TS-treated individuals crossed the respective TS apparatus less than the control fish, one could assume that it was a response to the putative visual and physical blockage created by the device. However, the apparatus did not appear to be strongly avoided, as fish continued to cross it throughout the experiment. For instance, during an efficiency test of a similar apparatus, Gauy et al. (2021) showed that Nile tilapia males were spontaneously choosing to pass through the bristles despite having the option to avoid them. This is probably the case of our study fish, which were passing through the apparatus without TS more frequently due to the ease of movement, as the silicone bristles may cause some resistance due to visual blocking. Future testing of this species' preferences could test for an apparatus that include only 50% of bristles and this way testing if individuals would still choose to pass through it.

Aggressive interactions seem to have been influenced by bristle enrichment. Individuals in the bristle group showed restrained aggressive behaviours (displays) but did not necessarily engage in significantly overt fights (attacks) while there was an interaction between the effect of treatment X time regarding the levels of attacks. This suggests that while bristles may have had an overall calming effect on their aggressive menacing, they did not eradicate their aggressive tendencies. A similar effect of aggression reduction has been reported in male Nile tilapia kept in isolation (Bolognesi et al., 2019), and in groups following a similar

Table 1
Two-way factorial ANOVAs, with treatment (TS, no TS) and stress confinement test (yes, no) as fixed factors, for cortisol and brain monoamines at 3 main brain regional sections (forebrain, midbrain and hindbrain). In bold are the significant results.

Sparus aurata		Control and Without stress	Control and With stress	TS and Without stress	TS and With stress	$F_{(1,59)}$	p
						C vs TS: 1.90	0.17
	Cortisol	19.51 ± 4.45	456.26 ± 85.96	27.83 ± 8.47	316.64 ± 38.44	Without vs With: 58.02	**<0.0001**
						Interaction: 2.41	0.12
						C vs TS: 0.18	0.67
	NA	774.88 ± 52.78	719.72 ± 38.13	736.51 ± 30.71	793.19 ± 39.01	Without vs With: 0.00	0.98
						Interaction: 1.86	0.18
						C vs TS: 3.23	0.07
	DOPAC	13.33 ± 0.96	16.76 ± 1.23	15.81 ± 1.54	19.04 ± 1.48	Without vs With: 6.29	**0.01**
						Interaction: 0.005	0.94
						C vs TS: 1.31	0.26
	DA	74.04 ± 3.36	84.75 ± 3.30	82.75 ± 4.00	84.06 ± 3.24	Without vs With: 2.94	0.09
						Interaction: 1.80	0.18
						C vs TS: 0.89	0.35
Forebrain	5-HIAA	326.12 ± 17.78	328.79 ± 19.55	324.76 ± 21.42	368.57 ± 22.40	Without vs With: 1.31	0.26
						Interaction: 1.02	0.31
						C vs TS: 0.38	0.54
	5-HT	929.44 ± 64.67	945.5 ± 49.63	881.41 ± 45.47	925.25 ± 60.21	Without vs With: 0.29	0.59
						Interaction: 0.06	0.8
						C vs TS: 1.48	0.23
	Ratio DOPAC/DA	18.15 ± 1.27	19.97 ± 1.45	19.06 ± 1.54	22.58 ± 1.50	Without vs With: 3.39	0.07
						Interaction: 0.34	0.56
						C vs TS: 0.84	0.36
	Ratio 5-HIAA/5-HT	37.85 ± 3.41	35.69 ± 2.10	37.38 ± 2.44	41.43 ± 2.43	Without vs With: 0.08	0.78
						Interaction: 1.20	0.28
						C vs TS: 0.55	0.46
	NA	553.74 ± 26.14	539.67 ± 25.18	551.4 ± 25.19	578.62 ± 21.38	Without vs With: 0.07	0.79
						Interaction: 0.70	0.4
						C vs TS: 0.63	0.43
	DOPAC	7.09 ± 0.33	8.47 ± 0.57	7.2 ± 0.60	9.17 ± 0.46	Without vs With: 10.96	**0.001**
						Interaction: 0.34	0.56
						C vs TS: 0.22	0.64
	DA	71.04 ± 3.29	70.95 ± 4.62	66.11 ± 4.68	71.99 ± 3.74	Without vs With: 0.49	0.49
						Interaction: 0.52	0.47
						C vs TS: 0.27	0.61
Midbrain	5-HIAA	132.24 ± 6.39	131.36 ± 9.17	130.98 ± 9.43	140.96 ± 6.67	Without vs With: 0.32	0.57
						Interaction: 0.45	0.5
						C vs TS: 0.27	0.6
	5-HT	519.28 ± 27.66	490.13 ± 31.03	489.33 ± 33.78	488.85 ± 25.60	Without vs With: 0.25	0.62
						Interaction: 0.23	0.63
						C vs TS: 0.29	0.59
	Ratio DOPAC/DA	10.23 ± 0.59	13.13 ± 1.78	11.19 ± 0.78	13.47 ± 1.27	Without vs With: 4.63	**0.03**
						Interaction: 0.07	0.8
						C vs TS: 1.36	0.25
	Ratio 5-HIAA/5-HT	25.82 ± 0.94	27.56 ± 1.55	26.88 ± 0.92	29.22 ± 1.11	Without vs With: 3.05	0.08
						Interaction: 0.07	0.79
						C vs TS: 0.57	0.45
	NA	604.05 ± 22.80	600.43 ± 15.84	593.37 ± 24.83	643.86 ± 22.15	Without vs With: 1.17	0.28
						Interaction: 1.56	0.22
						C vs TS: 0.07	0.79
	DOPAC	3.33 ± 0.26	4.04 ± 0.14	3.49 ± 0.36	4.03 ± 0.31	Without vs With: 4.89	**0.03**
						Interaction: 0.09	0.76
						C vs TS: 1.67	0.2
	DA	58.06 ± 2.32	59.93 ± 2.27	55.72 ± 2.55	56.10 ± 2.38	Without vs With: 0.22	0.64
						Interaction: 0.10	0.76
						C vs TS: 4.70	**0.03**
Hindbrain	5-HIAA	108.04 ± 4.82	109.76 ± 5.49	117.43 ± 6.31	128.24 ± 8.71	Without vs With: 0.95	0.33
						Interaction: 0.50	0.48
						C vs TS: 0.07	0.8
	5-HT	392.30 ± 21.51	392.97 ± 14.70	382.37 ± 15.84	412.81 ± 23.72	Without vs With: 0.66	0.42
						Interaction: 0.60	0.44
						C vs TS: 1.21	0.27
	Ratio DOPAC/DA	5.68 ± 0.32	6.86 ± 0.31	6.24 ± 0.57	7.33 ± 0.60	Without vs With: 5.82	**0.02**
						Interaction: 0.01	0.92
						C vs TS: 4.24	**0.04**
	Ratio 5-HIAA/5-HT	28.10 ± 1.08	28.28 ± 1.45	31.06 ± 1.80	31.33 ± 1.39	Without vs With: 0.02	0.87
						Interaction: 0.001	0.97

long-term protocol (21 days, Gauy et al., 2022). Following studies observed that these male tilapias exposed to the TS device increased growth as well as reduced the negative effects of high stocking density in this species, such as feeding efficiency and condition factor (Gauy et al., 2023; Gauy et al., 2022). Nevertheless, considering the relative variable effect of both time and treatment on *S. aurata* aggressive tendencies (displays and fights), future studies should also be focused on the individual (instead of solely group) tracking across treatment and time, to find out if other putative variables such size or dominance status may have a significant influence on their behavioural output and their

Table 2
Post hoc contrasts by planned comparisons.

Sparus aurata			$F_{(1,59)}$	p
Cortisol	C vs TS	Without stress	0.01	0.9
		With stress	4.23	**0.04**
	Without vs With	C	42.75	< **0.0001**
		TS	18.09	**0.00008**
DOPAC_forebrain	C vs TS	Without stress	1.78	0.19
		With stress	1.45	0.23
	Without vs With	C	3.4	0.07
		TS	2.91	0.09
DOPAC_midbrain	C vs TS	Without stress	0.02	0.88
		With stress	0.93	0.34
	Without vs With	C	3.78	0.06
		TS	7.46	**0.008**
Ratio DOPAC/DA_midbrain	C vs TS	Without stress	0.32	0.57
		With stress	0.04	0.84
	Without vs With	C	2.95	0.09
		TS	1.76	0.19
Ratio 5-HIAA/5-HT_midbrain	C vs TS	Without stress	0.41	0.52
		With stress	1.002	0.32
	Without vs With	C	1.12	0.29
		TS	1.98	0.16
DOPAC_hindbrain	C vs TS	Without stress	0.17	0.68
		With stress	0.0007	0.98
	Without vs With	C	3.22	0.08
		TS	1.79	0.19
5-HIAA_hindbrain	C vs TS	Without stress	1.08	0.3
		With stress	4.07	**0.04**
	Without vs With	C	0.04	0.85
		TS	1.39	0.24
Ratio DOPAC/DA_hindbrain	C vs TS	Without stress	0.73	0.39
		With stress	0.49	0.49
	Without vs With	C	3.21	0.08
		TS	2.63	0.11
Ratio 5-HIAA/5-HT_hindbrain	C vs TS	Without stress	2.09	0.15
		With stress	2.15	0.15
	Without vs With	C	0.008	0.92
		TS	0.02	0.89

interaction with the apparatus.

Regarding physiological stress responses measured by gill cortisol levels, it was clear that the confinement challenge was effective overall in inducing an acute stress. Interestingly, the effect of the bristle treatment was only more evident in individuals subjected to the confinement stress test, with those exposed to bristles showing lower cortisol response in a situation of acute stress, suggesting that the presence of bristles reduces or mitigates stress responses in this species. In fish, access to physical contact was first demonstrated to decrease stress in a reef species (the surgeonfish *Ctenochaetus striatus*, Soares et al., 2011) and to minimize fear and cortisol levels in zebrafish (Schirmer et al., 2013). But before it had been shown to reduce stress in other taxa, such as in humans (Field et al., 2005), beef cattle (Probst et al., 2013), horses (Kędzierski et al., 2017) and dogs (Rehn et al., 2013). In contrast to findings in surgeonfish *C. striatus* by Soares et al. (2011), access to physical stimulation was only found to have a mitigating effect in individuals subjected to the confinement stress test, suggesting that the stress-reducing benefits of bristle exposure may be reduced in baseline conditions (or perhaps take more time to become significant) but more meaningful in contexts acute stress. In fact, cortisol has been shown not to decrease in response to other types of enrichment, even when other welfare indicators are improved. For example, a bubble curtain, serving as a sensory, occupational, and physical enrichment, enhances cognitive performance, promotes growth, and reduces abnormal behaviours in rainbow trout fingerlings, yet it does not lower cortisol levels (Amichaud et al., 2024). Similarly, *S. aurata* exhibited a slight reduction in cortisol after 30 days of exposure to structural enrichment (suspended ropes), but this effect diminished over time, despite improvements in positive behavioural indicators (Oliveira et al., 2024). Conversely, a similar enrichment effectively mitigated husbandry stress in *S. aurata*, as evidenced by reduced heart rate and quicker recovery to baseline levels (Cabrera-Álvarez et al., 2024), as well as cognitive performance enhancement (Arechavala-Lopez et al., 2020). Our study is the first to show that exposure to physical enrichment is effective in situations of higher stress. Nevertheless, it is currently unknown whether increasing the time of bristle exposure would be successful in raising the impact of this sensorial enrichment in both baseline and surpassing conditions of higher stress.

In this study, we also wanted to investigate whether potential stress (confinement)- or enrichment (tactile-stimulation)-related effects on monoaminergic pathways would be able to show both a more generalized and/or regional patterns. Similarly to cortisol levels, the main driver of changes in dopaminergic activity (ratio DOPAC/DA) in *S. aurata* was the confinement test stress, which induced dopaminergic activation in the midbrain and hindbrain in particular. But beyond *S. aurata* response to acute stress, tactile enrichment was also associated with change in DOPAC levels across brain regions (see Table 1) but particularly at the midbrain, suggesting enhanced activation of dopaminergic pathways following 21 days of bristle introduction (see Table 2). Thus, it seems that bristle enrichment is involved in enhancing dopaminergic signalling, affecting structures involved in visual processing but also in integrating sensory and visual information, motivation, learning and memory, or in modulating different endocrine responses (O'Connell et al., 2010). Changes in the serotonergic system were mostly observed at the hindbrain, with the ratio of 5-HIAA/5-HT and 5-HIAA levels responding to the bristle treatment. Interestingly, hindbrain 5-HIAA levels were higher amongst those in the bristle treatment following confinement test (see Table 1), which suggests that this type of enrichment affects the 5-HT pathways in an area which function is connected to stress coping, behavioural inhibition and aggression changes, and sensory-motor modulation (Lillesaar, 2011; Winberg and Thörnqvist, 2016).

Overall, the results highlight crucial behavioural and physiological responses by seabreams *S. aurata* to physical/tactile stimulation. Individuals in the bristle enrichment group were less inclined to display aggression, and cortisol measurements further suggest that the bristle treatment modulated stress responses, with those exposed to bristles showing lower cortisol response in a situation of acute stress. Regarding brain monoaminergic activity, differences occurred at two main regional sections: midbrain and hindbrain, both involved in seabream response to acute stress and in connection with bristle enrichment. Our findings are the first demonstration that exposure to tactile stimulation during a limited amount of time is enough to produce behavioural changes, mitigate stress responses and modify brain monoaminergic signalling. However, it is important to emphasize that the present study was conducted under controlled laboratory conditions, which allowed us to isolate the effects of tactile stimulation on behaviour and physiology of our focus species. Given that there is currently very limited information on the responses of this species to tactile enrichment, this controlled approach was considered a necessary first step to determine whether such stimulation elicits measurable behavioural and physiological effects. Although this design provides strong experimental control, it does

not replicate the full complexity of commercial aquaculture systems, where factors such as stocking density, system size, water flow, oxygen gradients, feeding regimes, and routine management procedures may interact and influence fish responses. Therefore, the present findings should be interpreted as a proof-of-concept demonstrating that physical stimulation can influence seabream behaviour and stress physiology under controlled conditions, rather than as a direct recommendation for immediate application to aquaculture systems. Future studies should further ascertain if this type of enrichment may indeed have potential for aquaculture application, particularly in this important commercial species, the gilthead seabream *S. aurata*.

CRediT authorship contribution statement

Marta C. Soares: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ana Carolina dos Santos Gauy:** Writing – review & editing, Supervision, Software, Formal analysis. **Júlia Macieira:** Software, Methodology, Investigation. **Bianca Cambiaghi e Silva:** Methodology, Data curation. **Filipe Banha:** Writing – review & editing, Validation, Resources. **Manuel Gesto:** Writing – review & editing, Validation, Resources, Methodology. **Eliane Gonçalves-de-Freitas:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Conceptualization. **Margarida Saavedra:** Writing – review & editing, Supervision, Resources, Methodology, Investigation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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