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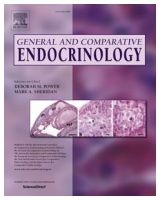

Nonapeptide modulation of looming-induced responses in male *Betta splendens*

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ABSTRACT

The looming stimulus is a widely used paradigm for investigating defensive behaviours in prey-predator contexts, as it simulates an approaching threat through the rapid expansion of a dark disk. Such stimuli typically evoke fight, flight, or freeze responses. In the present study, the role of the nonapeptides oxytocin and vasotocin, both known for their roles in social behaviour and stress regulation, in the behavioural and endocrine response to a looming stimulus in the Siamese fighting fish *Betta splendens* was assessed. Fish displayed pronounced freezing behaviour and the expression of conspicuous body stripes in response to a 10-min looming stimulus. Despite the marked behavioural reaction, no concomitant changes in plasma cortisol levels were recorded. The oxytocin receptor antagonist (L-368,899) did not affect the behavioural response, whereas treatment with the vasotocin receptor antagonist Manning compound enhanced fight and reduced flight responses. None of the nonapeptides modulated post-looming plasma cortisol levels. These findings provide new insights into the expression and regulation of stripe patterns in *B. splendens*, a phenomenon previously noted in aquaculture and behavioural studies but not quantitatively described, and highlight the involvement of the vasotocin system in the modulation of defensive strategies.

1. Introduction

In Nature, animals frequently encounter various types of threats, such as the presence of predators. Once a threat is detected, animals may enter a state of stress that activates defensive reactions to cope with the predator's presence. High-imminence, proximal threats provoke the "fight-flight-freeze" response. This response relies on what was first described in the "fight or flight" model by Cannon (1932), and later expanded to include the "freeze" response through the revised Reinforcement Sensitivity Theory by Gray and McNaughton (2000). These "fight-flight-freeze" responses are considered defensive fixed reaction patterns; unlike simple reflexes, they are less directly tied to stimulus intensity, have slower onset, and involve complex, coordinated behavioural patterns (Ledoux and Daw, 2018). In addition, studies in rodents have shown that these defensive behaviours are modulated by different brain areas, each involved in specific responses depending on threat proximity (reviewed in Tseng et al., 2023). The areas involved are part of structures such as the limbic system or the hypothalamus, specifically the paraventricular nucleus, the starting point of the hypothalamic-pituitary-adrenal axis (HPI axis), which initiates the stress response. This indicates that defensive behaviours and the stress response are closely linked, and when we refer to a defensive state and stress, we are addressing a complex, multicomponent condition modulated by several overlapping factors.

A key feature of this stress response is its regulation by neuropeptides, including oxytocin and vasotocin, in this paper referred to as OT and VT, respectively, following the suggestion by Theofanopoulou et al., 2021. OT and VT in fish are implicated in a wide range of functions. They have been principally studied in contexts related to social behaviour (e.g. reviewed in Kareklas et al., 2025), sexual behaviour and reproduction (Altmieme et al., 2019; Goncalves et al., 2024), as well as osmoregulation (Kulczykowska, 2019). In addition, both nonapeptides influence and modulate the stress response by acting directly on the HPI axis, and it is this role in stress modulation that the present paper investigates. OT exerts an anxiolytic effect, reducing both the activity and responsiveness of the HPI axis in a context-dependent manner (when the OT system is active) (Abramova et al., 2020; Jurek and Neumann, 2018). VT, on the other hand, influences pituitary adrenocorticotropic hormone (ACTH) and interrenal cortisol output, primarily activating the HPI axis (anxiogenic effect) (e.g. Balment et al., 2006; Gesto et al., 2014). In fish,

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the effects of OT on stress have been mainly investigated in social contexts, particularly its role in social buffering during stress tests (Akinrinade et al., 2023; Wilson et al., 2024), as well as in anxiety-like behavioural assays such as the open field and dark-light arena tests. While OT's role in social buffering is well established, its function during stress tests remains unclear. Most studies using antagonists or mutant lines report no effect of OT on anxiety-like behaviour (Gemmer et al., 2022; Landin et al., 2020; Ribeiro et al., 2020), although OT agonists have produced anxiolytic effects in zebrafish *Danio rerio* larvae (Maciag et al., 2025). Considering VT's role, for example, in rainbow trout *Oncorhynchus mykiss,* VT transcript levels increase in parvocellular, but not magnocellular, neurons (Gilchriest et al., 2000), supporting VT's direct involvement in ACTH secretion (Baker et al., 1996; Rivier and Vale, 1983). Braida et al. (2012) showed that nonapeptides and their antagonists modulate behavioural responses to predators in zebrafish in a dose-dependent manner, with nonapeptides decreasing and antagonists increasing fear responses, suggesting that these systems act in the same direction. Additionally, the anxiolytic drug diazepam reduced freezing behaviour in this context, indicating an anxious component in the observed response (Braida et al., 2012). Investigations of nonapeptide modulation of the stress responses have been conducted primarily in zebrafish (e.g. Braida et al., 2012; Maciag et al., 2025), with research on other species being limited (e.g. rainbow trout, Gilchriest et al., 2000). The species examined in this study, the Siamese fighting fish *Betta splendens* contrasts with the highly social zebrafish in being notoriously solitary, as males actively defend territories from other males, with the two species diverging for more than 100 million years (Zhang et al., 2022). This provides an opportunity to investigate the role of OT and VT in the modulation of the stress response across fish species with divergent social systems and that are phylogenetically distant.

In fish, several experimental paradigms have been used to simulate the presence of predators. Two of the most common predation-related stressors are alarm substances, chemical compounds released by some fish species in the presence of threats to signal danger to others, and the looming stimulus, a sudden appearance of a shape to mimic the shadow or the presence of a predator. Alarm substances represent a socially mediated form of threat detection, as they are part of a chemical communication system. However, the existence of alarm substances has been demonstrated in only a few species, including the Nile tilapia *Oreochromis niloticus*, convict ciclid *Amatitlania nigrofasciatus,* frillfin goby *Bathygobius soporator* (reviewed in Maximino et al., 2019), zebrafish (Speedie and Gerlai, 2008), and other Cyprinids (Døving et al., 2005). The looming stimulus is highly reproducible due to the possibility of controlling its appearance and mode of presentation, allowing for cross-species comparisons under the same experimental paradigm. Furthermore, it represents a purely visual input, making it more suitable for visual-guided species such as the Siamese fighting fish *B. splendens*. Considering that alarm substances have so far not been described for this species, the looming stimulus was selected as a predation-related stressor. Further, although no published description exists about the natural predators of *B. splendens*, their habitat in shallow and still water bodies suggests that aerial predators like birds, simulated by the looming stimulus, may be common (for a description of the habitat, see Ramos et al., 2025).

The looming stimulus is typically presented in two dimensions, often on a screen, mimicking the approach of an object that may represent a predator. Responses to the looming stimulus have been studied and characterised across multiple taxa, including insects (Fotowat and Gabbiani, 2007; Holmqvist, 1994; Zacarias et al., 2018), fish (Dill, 1974; Preuss et al., 2006), crustaceans (Oliva and Tomsic, 2012), reptiles (Hayes and Saiff, 1967), amphibians (King et al., 1999), birds (Sun and Frost, 1998; Tronick, 1967), mammals (Cruz et al., 2020; Schiff et al., 1962; Yilmaz and Meister, 2013), and humans (Ball and Tronick, 1971; Bower et al., 1971). One of the most common responses to the looming stimulus is a fast-start escape (Bhattacharyya et al., 2017; Dunn et al., 2016; Preuss et al., 2006; Temizer et al., 2015), with a dark expanding disk being particularly effective in eliciting this reaction in zebrafish larvae (Temizer et al., 2015).

Beyond overt behaviours such as freezing or escape, Siamese fighting fish also exhibit pronounced colour changes during stress response, commonly referred to as “stress stripes”. While previously described in aquaculture settings, these stripes have never been systematically studied/characterised or quantified. A few articles mention stripes in various contexts, but never consider them as the primary research focus. For example, Robertson and Sale (1975) proposed the use of a model with longitudinal stripes as a submissive male, without further analysis or reference, while Braddock and Braddock (1955) observed stripes in a pre-fight context but did not explore the phenomenon further. The looming stimulus provided a tool to study this phenomenon in further detail.

Given this background, our study aimed to characterise the behavioural response of *B. splendens* to the looming stimulus, a tool commonly used to mimic imminent threat or predator approach in a laboratory context and known to induce defensive behaviour and stress response. In addition, we investigated the potential effects of OT and VT on the stress response to a looming stimulus by administering receptor antagonists for both nonapeptides. As this is the first study to explore these questions in the Siamese fighting fish, particular attention was given to the analysis of the behavioural response, including a quantitative assessment of stress stripe expression.

2. Materials and methods

2.1. Animals and housing

The subjects used in the experiment were male *B. splendens*, aged between 26 and 40 months, from the F2 generation of a cross between a wild-type and a fighter strain (for details on this line, see Ramos et al., 2021). The rationale of using F2 hybrids was to capture potential variation in the OT and VT system across different lines of the species, considering that these strains already showed to differ in the expression of behaviour and endocrine response when tested in other contexts, such as aggression (Ramos and Gonçalves, 2019, 2022). Prior to the experiment, fish were kept in individual tanks (9 W × 9D × 20H cm) with shelters and were visually isolated from conspecifics for at least 48 h. Fish were fed twice daily: commercial dry food (Atison's Betta Pro) in the morning and live food (adult artemia) in the afternoon. No food was provided on the day of the experiment. Tank conditions, both for grouped and isolated fish, were kept constant, with a temperature of 28 ± 1 °C, a 12:12 h light:dark photoperiod, and water supplied by reverse osmosis (RO) with salinity maintained at 250 ppm.

2.2. Experimental setup – Baseline

The baseline experiment aimed to assess the behavioural and endocrine response of fish to the looming stimulus without nonapeptide manipulation. The test chamber consisted of a 25 W × 12.5D × 20H cm tank illuminated by a diffuse LED panel. Two Raspberry Pi camera modules (V2; one side view and one top view; 1640 × 922 px, 30 fps), each connected to an independent Raspberry Pi 4B board, allowed recording the trial. An LCD screen was positioned on the short side of the tank and remotely controlled via a Raspberry Pi board, displaying a white background during acclimation and recovery periods, and the looming stimulus video during the test phase (Fig. 1).

Fish were assigned to four different groups: Looming ($n = 8$), Control (n = 8), Looming-Recover ($n = 9$), and Control-Recover (n = 9). There were no significant differences in weight (W) or standard length (SL) in fish from different groups (One-way Anova, W: $F_{(3,30)} = 0.4166$, $p = 0.7423$; SL: $F_{(3,28)} = 0.2962$, $p = 0.8278$). All groups underwent a 60-min acclimation period in the test tank (25 W × 12.5D × 20H cm). This was followed by a 10-min test phase where fish in the Looming and Looming-Recover groups were exposed to the looming stimulus (three

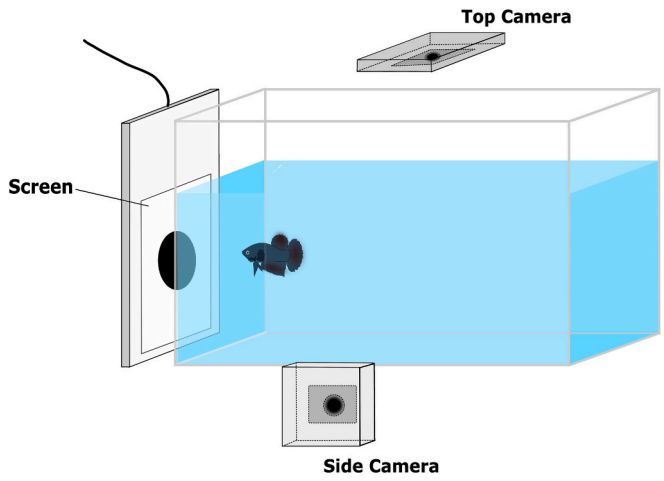

Fig. 1. Experimental set-up representation. After an acclimation phase, a looming stimulus was triggered on a screen in non-control groups, displaying a white background otherwise. The behaviour was recorded with both a top and a side camera. Illumination was provided by a diffuse LED strip.

random presentations per minute, each consisting of an approximately 0.5 s expansion period, during which the circle rapidly expanded to cover the entire display), whereas those in the Control and Control-Recover groups received no stimulus. The experiment was terminated for the Looming and Control fish, while Looming-Recover and Control-Recover groups experienced an additional 30-min recovery period after the test phase. Behavioural experiments were conducted between 10:40 and 19:00. Fish were distributed to time slots using a balanced design, such that each group was tested a comparable number of times in each time period.

At the end of the experiment, fish were anaesthetised with buffered MS222 (400 mg/L), weight and standard length were measured, and blood (approximately 10 µL) was collected from the caudal vein using a heparinised 27G syringe. Blood samples were centrifuged for 15 min to obtain plasma, which was transferred to a new clean tube and stored at −20 °C for hormonal analysis. After blood collection, animals were recovered and returned to their home tanks.

2.3. Experimental setup – OT and VT receptor antagonists experiment

Fish were randomly assigned to seven treatments that consisted of intraperitoneal injections with one of the following compounds: saline solution (0.9% NaCl in Milliq water, control group, $n = 9$); the OT receptor (OTR) antagonist L-368,899 hydrochloride (CAT#2641; CAS 1603112–62-9; Tocris Bioscience) at 1 µg/gbw (g body weight) (L1-low dosage, $n = 11$), 3 µg/gbw (L3-medium dosage, $n = 10$), 5 µg/gbw (L5-high dosage, n = 10); and the VT receptor (VTR) antagonist Manning compound (CAT#V2255; CAS 73168–24-8; Sigma) at 1 µg/gbw (M1-low dosage, $n = 8$), 3 µg/gbw (M3-medium dosage, n = 10), 5 µg/gbw (M5-high dosage, n = 10). Each fish was weighed prior to injection to ensure the volume administered was adjusted according to body weight. There were no significant differences in weight (W) or standard length (SL) between groups (Kruskal-Wallis test, W: $H(6) = 2.140$, $p = 0.9063$; SL: $H(6) = 2.045$, $p = 0.9156$). Injections were performed using a 1 mL syringe (BD). The choice of antagonists and concentrations was based on literature review. L-368,899 is a non-peptidergic antagonist that inhibits the OTR in mammals, leading to significant behavioural changes (Boccia et al., 2007), and has a higher affinity for the OTR than for the VTR (Landin et al., 2020; Manning et al., 2012). Additionally, as an unbiased antagonist, L-368,899 blocks OTR signalling through both Gq and Gi pathways (Williams et al., 2020). Dosages were selected after testing the concentrations proposed by Landin et al. (2020) for zebrafish (5, 50 and 100 µg/g body weight). In *B. splendens,* doses of 50 and 100 µg/g resulted in mortality, so 5 µg/g body weight was chosen as the highest dosage, with 3 µg/g body weight and 1 µg/g body weight as medium and low doses, respectively. The Manning compound is a widely used VTR1a receptor antagonist in research, known to have almost no antidiuretic activity (Guillon et al., 2004) and to block the VT signalling in teleosts, producing observable behavioural effects (Mahlmann et al., 1994; Semsar et al., 2001; Semsar and Godwin, 2004; Yaeger et al., 2014). In the aforementioned articles, a dosage of 3.2 µg/g body weight was used. Since there were no previous reports of antagonist use in *B. splendens*, doses of 1, 3, and 5 µg/g body weight were selected as low, medium, and high, respectively.

The experimental setup was as previously described (Fig. 1). All fish underwent a 60-min acclimation period, a 10-min looming stimulus exposure phase, and a subsequent 30-min recovery period. Behavioural experiments were conducted between 09:00 and 17:00. Fish were distributed to time slots using a balanced design to ensure that the different treatments were tested throughout the day, following the same approach used in the baseline experiment. After recovery, fish were anaesthetised with buffered MS222 (400 mg/L), measured, and blood (approximately 10 µL volume) was collected from the caudal vein using a heparinised 27G syringe. Blood collection for hormonal analysis was performed as previously described, after which the animals were allowed to recover and were returned to their home tanks.

2.4. Behavioural observation

For each fish, data from the two cameras was combined and in-house deep-learning software applied to extract variables related to the 3D position in the tank, including time spent within 5 cm of the stimuli, within 4 cm of the surface or bottom, and total distance travelled. The three spatial coordinates (x, y, z), extracted for each time frame, were used to compute a time-binned analysis of the distance travelled every 10 min. Aggressive display was quantified through the only two aggressive behaviours observed: time with opercula open (measured using the in-house software) and time with distended fins (measured manually with BORIS; Friard and Gamba, 2016). The duration of immobility, defined as no movement except for the eyes and gills, and erratic bursts, consisting of sharp changes in direction or velocity (Kalueff et al., 2013), was also registered manually using BORIS. To quantify stripe appearance, we measured the variance of grayscale pixel intensity in a fixed square located in the middle of the body using Fiji (Schindelin et al., 2012). Stripes were measured from the side view at the midpoint frame of each time period/bin, with the fish positioned laterally toward the side-view camera. It is important to note that both vertical and longitudinal stripes were observed and recorded. The variance of grayscale pixel intensity here is intended as a measure of overall change in colour pattern (from homogeneous to heterogeneous), without addressing the functionality of stripe orientation. As *B. splendens* is a labyrinth fish capable of air breathing, the number of breaths (air gulps) was manually counted using BORIS as an indicator of metabolic effort (Alton et al., 2012).

2.5. Hormonal analysis

Plasma cortisol concentration was measured with competitive enzyme-linked immunosorbent assay (ELISA) kits (Cayman Chemical), following the manufacturer's instructions. Ramos et al., 2021 previously confirmed the absence of interference in this species and with these ELISA kits by performing serial dilutions of a plasma pool and comparing the resulting slope to that of a standard curve. All standards and samples were measured in duplicate with a 1:20 dilution in EIA buffer. To analyse all samples, three assay runs were required. The intra-assay coefficient of variation, calculated from the duplicate samples, was 4.5% for the baseline groups. The samples from the groups treated with the nonapeptide receptor antagonists were divided as follows in two assays: assay 1, C $n = 5$, L1 n = 5, L3 n = 5, L5 n = 5, M1 n = 5, M3 n = 5,

M5 $n = 6$, and assay 2, C $n = 4$, L1 n = 6, L3 n = 5, L5 n = 5, M1 $n = 3$, M3 n = 5, M5 n = 4, with an inter-assay coefficient of variation of 4.9% and 7.6% respectively.

2.6. Data analysis

All data were tested for normality and homoscedasticity using Shapiro-Wilk's and Levene's tests, respectively. If parametric assumptions were violated, variables were log-transformed and retested. When the transformation did not resolve violations, appropriate non-parametric tests were applied. The analysis performed will be described separately for each parameter, with first a description of the baseline experiment and then the antagonist receptor experiment.

Factor analysis. Factor analysis was conducted separately for the baseline and receptor antagonist treatment experiments, using the following variables: aggressive display (total duration of threat display, including opercular opening and fins distention), time spent immobile, total duration of erratic bursts, distance travelled, time spent near the test area, time spent near the surface, time spent near the bottom, presence of stripes (grey pixel standard deviation), and number of surface air breathing events. Factor analysis was performed using the R factanal() function, with three factors, estimated according to the method proposed by Morton and Altschul (2019), and varimax rotation, which is recommended for smaller sample sizes (Budaev, 2010).

After the extraction of factor scores, for the baseline experiment, comparisons between the two main test types (Control vs Looming) during the test phase were performed using Welch's *t*-test or Wilcoxon signed-rank test, depending on data distribution assumptions. Since the four baseline test types did not differ during the test phase, they were analysed together. Effect sizes were calculated as Cohen's *d* for *t*-tests and *r* (Wilcox effect size) for Wilcoxon tests.

For the receptor antagonist treatment, Kruskal-Wallis tests were used (due to non-parametric data) to assess dosage effects, with rank epsilon squared for effect size. To examine treatment, factor, and interaction effects, a nonparametric analysis using the aligned rank transform (ART) procedure was performed using the *ARTool* (*v0.11.2*; Kay et al., 2025) package in R, followed by pairwise comparisons within each treatment (Dunnett's test against control) and calculation of Cliff's delta for effect size.

Time-binned distance travelled. Time-binned analysis on distance travelled every ten minutes was calculated for both baseline and receptor antagonist injection experiments.

For the baseline, the two looming groups and the two control groups were combined, and a linear mixed model was used to test for effects of test type (Control vs. Looming), test phase (PRE10, PRE20, PRE30, TEST, POST10, POST20, POST30), and their interaction. Subject ID was included as a random intercept. Effect sizes (partial eta squared) and marginal/conditional R^2 values are reported. Pairwise comparisons of test type groups were conducted within each test phase (comparing Control vs. Looming). In addition, planned comparisons of selected time points were performed within each test type to assess the differences between consecutive test phases, as well as between each PRE and POST phase relative to TEST.

For the receptor antagonist injection experiment, a linear mixed model was used with fixed effects for treatment (C, L, M as no effect was observed between dosages on the behavioural measures) and test phase (PRE10, PRE20, PRE30, LOOMING, POST10, POST20, POST30) with subject ID as a random effect. Effect sizes (partial eta squared) and marginal/conditional R^2 values are provided. Planned comparisons of selected time points, including consecutive test phases, as well as each PRE and each POST phase relative to LOOMING, were performed to assess temporal changes, ignoring treatment group, as the interaction between treatment and time was not significant.

Stripes. Stripe presence in the baseline experiment was analysed using a linear mixed model to assess the effects of test type (Control vs. Looming), test phase (pre, test, post), and their interaction, with subject ID as a random intercept. Partial eta squared and marginal/conditional R^2 values are reported. Pairwise comparisons for treatment within each timepoint and for timepoint within each treatment were performed with Tukey adjustment.

For the receptor antagonist injection experiment, during the looming phase, a one-way ANOVA was conducted to test for treatment effects on stripe appearance, with partial eta squared as effect size. As treatment did not have a significant effect, all data were combined to examine changes in stripe appearance across phases using a Kruskal-Wallis test, followed by Dunn's post hoc test with Holm correction and eta squared for effect size.

Hormonal analysis. For baseline cortisol analysis, a two-way ANOVA examined the effects of test type (Control vs. Looming), sampling time (10 min post-test or 30 min after the recovery phase), and their interaction, reporting partial eta squared and R^2.

For the receptor antagonist treatment, two one-way ANOVA assessed the effects of treatment and dosage, with eta squared as effect size.

Immobility. Immobility during recovery in the receptor antagonist experiment was analysed using Welch's ANOVA due to unequal group sizes and variances, with eta squared as the effect size.

Statistical analyses were performed using R Statistical Software v4.3.0 (R Core Team, 2021). Graphical representations were carried out using GraphPad© Prism (v8.4.0; GraphPad Software LLC, San Diego, CA), and figures were edited and completed using Inkscape© (v. 1.2.2; Free Software Foundation Inc.).

2.7. Ethical note

All methods adhered to the ASAB/ABS ("Guidelines for the treatment of animals in behavioural research and teaching", (Anim. Behav., 2012)). This study followed the ethical guidelines enforced at the Institute of Science and Environment of the University of Saint Joseph, and work with the species is approved by the Division of Animal Control and Inspection of the Civic and Municipal Affairs Bureau of Macau, license AL017/DICV/SIS/2016.

3. Results

3.1. Baseline

Factor analysis. The factor analysis (KMO = 0.59, Bartlett's: $\chi 2 = 219.08$, $p < 0.001$) extracted 3 factors: factor 1 exhibited significant loadings (> 0.4) for erratic burst and distance travelled, representing the flight response; factor 2 loaded highly (> 0.4) on immobility and on stripes, which we interpret as the freeze response; and factor 3 showed strong loadings (> 0.5) for aggressive display and time spent near the surface, corresponding to the fight response (Table 1 - Refer to the Supplementary Video 1 for illustrative videos). Fish exposed to the looming stimulus showed a higher level of freezing response (Wilcoxon signed-rank test $p < 0.0001$, $r = 0.784$) compared to the control fish;

Table 1
Loadings extracted from the factor analysis of behaviours during the baseline experiment. Significant loadings that contributed to the definition of the different factors are highlighted in bold.

Loadings	Factor 1	Factor 2	Factor 3
Aggressive Display	−0.131	0.131	**0.560**
Immobility	−0.186	**0.677**	−0.360
Erratic Burst	**0.991**	0.079	0.082
Distance travelled	**0.853**	−0.495	0.153
Time near Test	0.087	−0.099	0.375
Time near Surface	**0.458**	−0.476	**0.698**
Time near Bottom	−0.387	**0.414**	−0.821
Stripes	0.032	**0.549**	0.034
Air breaths	0.176	−0.734	0.133
	Flight	**Freeze**	**Fight**

however flight (Wilcoxon signed-rank test $p = 0.118$, $r = 0.276$) and fight ($t(30.7) = -0.53$, $p = 0.601$, $d = -0.184$) behaviours did not differ between the two test types (Fig. 2A).

Time-binned distance travelled. The time-binned analysis for the distance travelled was computed excluding the three high-activity individuals because they disproportionately influenced the variance structure, obscuring the overall pattern of the data. The results showed a significant main effect of test phases ($F_{(6,\ 128.94)} = 9.46$, $p < 0.001$, $\eta^2_p = 0.66$), but no significant main effect of test type ($F_{(1,\ 30.23)} = 0.01$, $p = 0.915$, $\eta^2_p = 0.0004$). Importantly, there was a significant test type × test phase interaction ($F_{(6,\ 128.94)} = 7.88$, $p < 0.001$, $\eta^2_p = 0.609$), indicating that the effect of the looming stimulus varied across different test phases; conditional and marginal R^2 were respectively 0.549 and 0.282. Post hoc comparisons within test phases revealed that Control and Looming differed between them only during the TEST phase (estimate = 44.882, SE = 8.78, t-ratio = 5.111, $p < 0.0001$). All the other test phases showed no differences (see Table 1 in Supplementary Material for all planned comparisons). Planned comparison within test type showed that control individuals did not vary in the distance travelled across the different time phases, except for TEST and POST10 phases (see Table 2A in

Table 2
Loadings extracted from the factor analysis of behaviours during the receptor antagonist experiment. Significant loadings that contributed to the definition of the different factors are highlighted in bold.

Loadings	Factor 1	Factor 2	Factor 3
Distance travelled	−0.261	**0.923**	0.054
Time near Surface	−0.993	0.064	−0.067
Time near Bottom	**0.997**	−0.118	−0.095
Aggressive Display	−0.182	0.099	**0.837**
Erratic Burst	−0.250	**0.818**	−0.231
Time near Test	0.009	0.348	−0.026
Air breaths	−0.620	0.387	−0.261
Immobility	**0.406**	−0.374	−0.171
Stripes	−0.102	0.112	−0.270
	Freeze	**Flight**	**Fight**

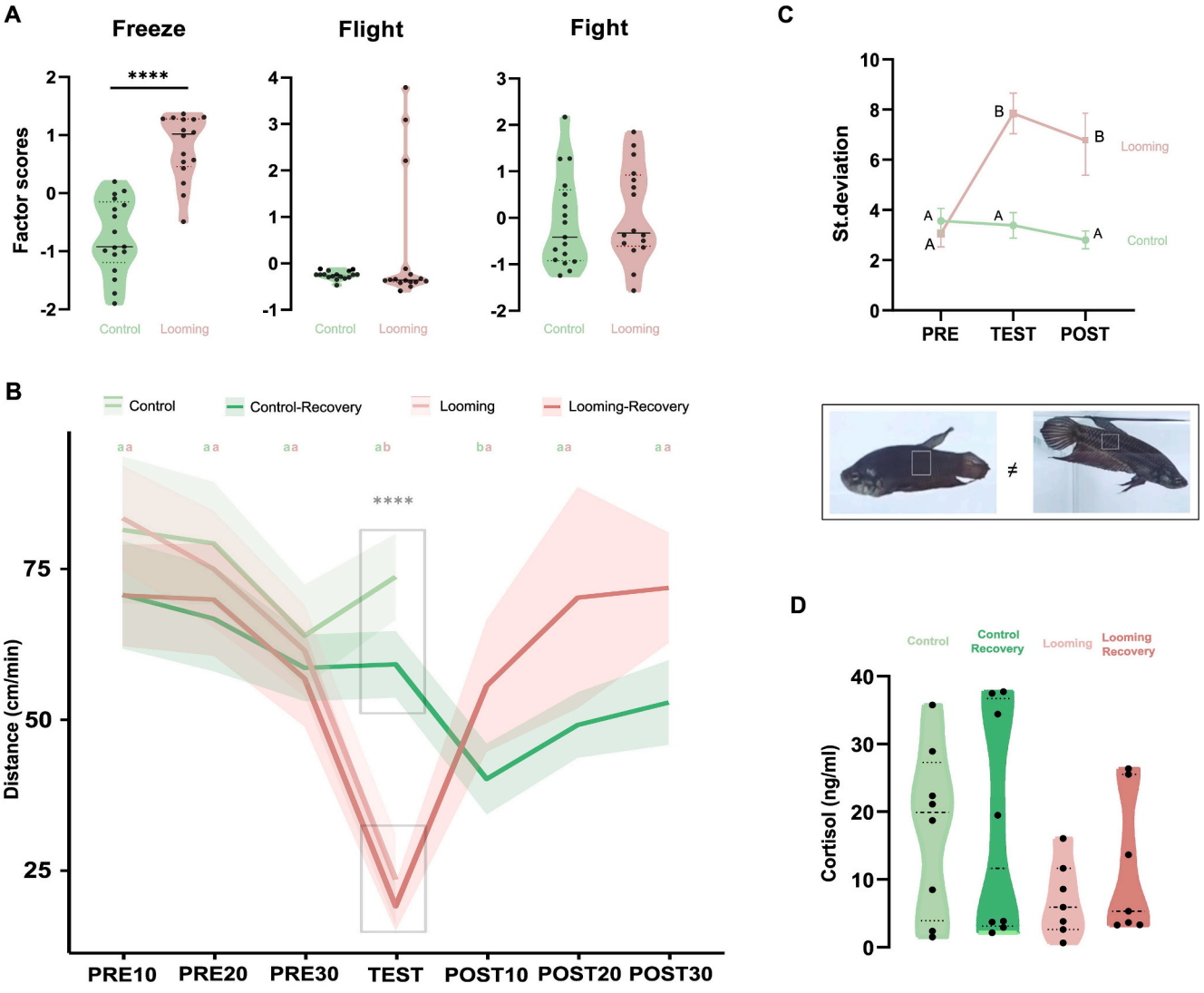

Fig. 2. Behavioural and physiological responses of *B. splendens* under Control and Looming conditions. (A) Behavioural factors analysed in Control and Looming groups. Significance levels are indicated as ≤0.0001 (****). (B) Distance travelled every 10 min across all four test types. Statistical analysis was conducted only for the test phases by combining the two Control and the two Looming groups. Letters indicate differences across test phases within Control (green) and Looming (red). Asterisks represent the difference between Control and Looming groups during the test phase ($p \leq 0.0001$, ****). (C) Standard deviation of pixel intensity for the two test types (Control and Looming) measured across test phases. An example is shown below, illustrating the square used for measuring standard deviation, with visible body horizontal stripes in the fish on the right. (D) Plasma cortisol levels sampled at the end of the trials. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Supplementary Material for all planned comparisons). Meanwhile, regarding the Looming group, the TEST phase showed lower distance travelled compared to the PRE phases and the POST phases (Fig. 2B) (see Table 2B in Supplementary Material for all planned comparisons).

Stripes. Treatments ($F_{(1,33.61)} = 9.84$, $p = 0.004$, $\eta^2_p = 0.227$), test phase ($F_{(2,51.10)} = 8.55$, $p = 0.0006$, $\eta^2_p = 0.337$), and their interaction ($F_{(2,51.10)} = 10.5$, $p = 0.0002$, $\eta^2_p = 0.381$) all had significant effects on the appearance of stripes (conditional $R^2 = 0.401$, marginal $R^2 = 0.164$). In the control group, no significant differences were observed among test phases: PRE vs TEST (estimate = 0.038, SE = 46.3, t-ratio = 0.234, $p = 0.97$); PRE vs POST (estimate = 0.079, SE = 0.205, t-ratio = 0.384, $p = 0.92$); and TEST vs POST (estimate = 0.041, SE = 0.205, t-ratio = 0.197, $p = 0.98$). In contrast, individuals subjected to the looming treatment showed a significantly higher standard deviation of grey pixels intensity on the side, reflecting the presence of stripes, during both the TEST (estimate = − 0.099, SE = 0.169, t-ratio = −5.891, $p < 0.0001$) and POST phases (estimate = −0.76, SE = 0.216, t-ratio = −3.524, $p = 0.0025$) compared to the PRE phase. There was no significant difference between the TEST and POST phases (estimate = 0.24, SE = 0.216, t-ratio = 1.088, $p = 0.523$), indicating that the fish did not come back to their normal colouration during the recovery period (Fig. 2C).

Hormonal analysis. Neither treatment ($F_{(1,\ 27)} = 0.76$, $p = 0.39$, $\eta^2_p = 0.0027$), moment of collection ($F_{(1,27)} < 0.01$, $p = 0.99$, $\eta^2_p < 0.001$), nor their interaction ($F_{(1,27)} = 0.14$, $p = 0.71$, $\eta^2_p = 0.005$) had a significant effect on cortisol levels ($R^2 = 0.03$) (Fig. 2D).

3.2. OT and VT receptor antagonists experiment

Factor analysis. The factor analysis (KMO = 0.63, Bartlett's: $\chi 2 = 344.71$, $p < 0.001$) extracted 3 factors: factor 1 exhibited significant loadings (> 0.4) for time spent near the bottom and immobility, representing the freeze response; factor 2 loaded highly (> 0.5) on distance travelled and erratic burst, which we interpret as the flight response; and factor 3 showed strong loadings (> 0.5) for aggressive display corresponding to the fight response (Table 2). Factor loadings for factors 1 and 3 were sign-adjusted so that positive loadings consistently indicate greater expression of the defining behaviours. Dosages did not affect any of the three factors analysed: freeze (Kruskal-Wallis chi-squared = 3.55, *d.f.* = 6, $p = 0.737$, $\eta^2 = 0.06$, 95% CI = [0.06,1.00]; flight (Kruskal-Wallis chi-squared = 6.0625, *d.f.* = 6, $p = 0.416$, $\eta^2 = 0.11$, 95% CI = [0.09,1.00]; and fight (Kruskal-Wallis chi-squared = 4.7052, *d.f.* = 6, $p = 0.582$, $\eta^2 = 0.08$, 95% CI = [0.06, 1.00] (Fig. 3A). In the model analysis, neither the main effect of treatment ($F_{(2,\ 53)} = 2.28$, $p = 0.11$) nor the variable ($F_{(2,\ 106)} = 0.80$, $p = 0.45$) was statistically significant. However, there was a significant interaction between treatment and variable ($F_{(4,\ 106)} = 4.98$, $p = 0.001$), indicating that the effect of treatment depended on the specific behaviour measured. Specifically, injection with the Manning compound significantly increased the fight response compared to the saline control (estimate = −60.4, $p = 0.0499$, Cliff's delta = −0.41, 95% CI = [−0.77, 0.14]), and decreased the flight response relative to the saline (estimate = 65.8, $p = 0.029$, Cliff's delt = 0.5, 95% CI = [−0.29, 0.88]) (Fig. 3B).

Time-binned distance travelled. The three treatments analysed (C, L and M) did not differ in the distance travelled ($F_{(2,65)} = 0.7869$, $p = 0.46$, $\eta^2_p = 0.024$) at any of the test phases. However, the test phase had a significant effect on the distance travelled ($F_{(6,\ 390)} = 12.94$, $p < 0.0001$, $\eta^2_p = 0.544$), as also observed in the baseline experiment. There was no significant interaction between treatment and test phase ($F_{(12,390)} = 0.83$, $p = 0.62$, $\eta^2_p = 0.132$). During the looming stimulus, fish decreased their distance travelled compared to the PRE phases (PRE10 – LOOMING: estimate = 32.106, SE = 5.04, t-ratio = 6.374, $p < 0.0001$; PRE20 – LOOMING: estimate = 27.305, SE = 5.04, t-ratio = 5.421, $p < 0.0001$; PRE30 – LOOMING: estimate = 26.319, SE = 5.04, t-ratio = 5.225, $p < 0.0001$) and to the later POST phases (LOOMING – POST20: estimate = −27.511, SE = 5.04, t-ratio = −5.462, $p < 0.0001$; LOOMING – POST30: estimate = −25.618, SE = 5.04, t-ratio = −5.086, $p < 0.0001$). Unlike in the baseline, the distance travelled during the looming stimulus and the first 10 min of the recovery phase did not differ (estimate = −3.924, SE = 5.04, t-ratio = −0.799, $p = 0.987$) (see Table 3, Supplementary Material for all the planned comparisons). The conditional R^2 was 0.401, and the marginal R^2 was 0.164 (Fig. 3C).

Immobility. Treatments had no significant effect on the immobility levels during the recovery phase ($F_{(2,22.15)} = 2.83$, $p = 0.08$, $\eta^2 = 0.09$, 95% CI = [0.00,1.00] (Fig. 3D).

Stripes. During the looming test, treatments had no effect on stripes' appearance ($F_{(2,54)} = 0.55$, $p = 0.58$, $\eta^2 = 0.02$, 95% CI = [0.00,1.00]) (Fig. 2E). However, the test phase itself significantly influenced the fish colouration (Kruskal-Wallis chi-squared = 28.50, *d.f.* = 2, $p < 0.001$, $\eta^2 = 0.14$) as observed in the baseline experiment. All pairwise comparisons between test phases were statistically significant: PRE vs LOOMING ($z = 5.33$, $p < 0.001$), POST vs LOOMING ($z = 2.67$, $p = 0.0038$), PRE vs POST ($z = 2.75$, $p = 0.006$) (Fig. 1, Supplementary Material).

Hormonal analysis. Neither dosages ($F_{(4,60)} = 0.36$, $p = 0.836$, $\eta^2 = 0.009$, 95% CI = [0.00,1.00]) nor treatment ($F_{(2,60)} = 0.29$, $p = 0.749$, $\eta^2 = 0.02$, 95% CI = [0.00,1.00]) affected plasma cortisol levels measured after the recovery phase from the looming stimulus (Fig. 3F).

An additional analysis was conducted to assess whether age influences the cortisol response in the looming paradigm. For this analysis, older ($n = 15$) and younger ($n = 9$) animals were compared. These sample sizes are smaller than those reported in the main experiments, as only animals that underwent the looming stimulus without any antagonist receptor injection were included. No significant difference was observed between the two groups ($t(22) = -0.7824$, $p = 0.4423$, $d = -0.33$; data not shown).

4. Discussion

When subjected to a looming stimulus, most individuals exhibited a pronounced freezing response, which led to reduced swimming activity. This response was accompanied by the appearance of vertical and/or longitudinal stripes on the lateral part of the body, which did not fade even 30 min after the looming stimulus was removed, indicating that the fish did not fully recover to their initial state. Our results diverge to some extent from previous fish studies using the looming stimulus. Other studies have focused on measuring only the escape response and the neuronal network underlying it (Bhattacharyya et al., 2017; Dunn et al., 2016; Temizer et al., 2015), or on the kinematic interactions between prey and "predator" (Domenici, 2002; McKee and McHenry, 2020), rather than addressing the physiological stress response elicited by the looming test. Addressing the fight-flight-freeze response in *B. splendens*, our results revealed a clear difference between control and looming treatments only in the freeze component. In fact, no significant differences in escape (flight) and aggression (fight) behaviours were recorded between looming-exposed and control animals. Moreover, the motility of the animals returned to pre-test values within 10 min after the looming period ended.

Regarding the receptors' antagonist for OT and VT, no dosage-dependent effects were observed on the fight-flight-freeze response. However, treatment with the Manning compound prior to the looming stimulus led to a decrease in the flight response and an increase in the fight response, while the freeze response remained unchanged. Treatment with L-368,899 did not produce any significant effects. Neither compound altered distance travelled, immobility during recovery, or plasma cortisol levels at any tested dosage. The actions of OT and VT on the HPA axis are well documented in mammals (Mbiydzenyuy and Qulu, 2024; Satao and Doshi, 2024; Uvnäs-Moberg et al., 2024). In fish, in vitro studies have demonstrated their effects on corticotropin-release hormone (CRH) release; VTR expression is found in the pituitary pars distalis, where ACTH cells reside (Rawat et al., 2019), while OTR so far has only been linked to luteinizing hormone (LH) cells (Yang et al., 2021). Nevertheless, OT projections reaching the anterior pituitary

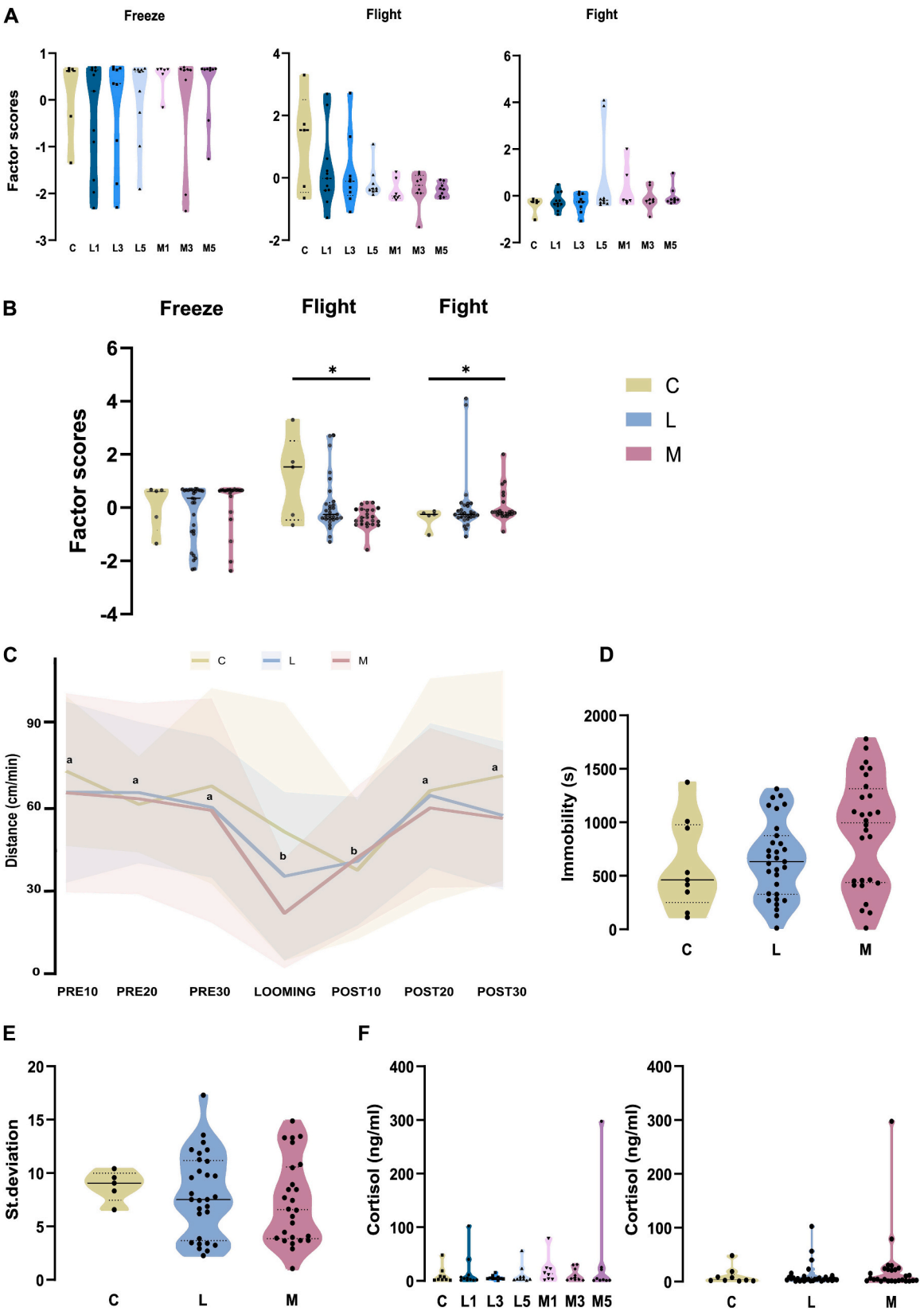

Fig. 3. Behavioural and physiological responses of *B. splendens* across dosages and treatments with OT and VT receptor antagonists. (A) Behavioural factors analysed by dosage and (B) by treatment. Because no differences were found among dosages, data were combined for further analysis. Significance level are indicated as ≤0.05 (*). (C) Distance travelled every 10 min across all three treatments. Different letters indicate significant differences among test phases. (D) Immobility rate during the recovery phase in the three treatments. (E) Standard deviation of pixel intensity for the three treatments measured during the looming phase. (F) Plasma cortisol levels analysed by dosage and treatment (F). For all panels, C represents the Control group (saline solution), L the group injected with L-368,899, and M the group injected with the Manning compound.

near CRH receptor sites have been shown (Batten et al., 1999). Additionally, there is evidence for bidirectional interactions between stress and the nonapeptide systems. Stressors can alter OT and VT levels in both brain and plasma, indicating activation of these systems in response to stress. For instance, increases in OT and VT have been observed in sea bream *Sparus aurata* after air exposure (Mancera et al., 2008; Skrzynska et al., 2018), environmental salinity change (Martos-Sitcha et al., 2013, 2014), food deprivation (Mancera et al., 2008; Skrzynska et al., 2017, 2018), and high stocking density (Mancera et al., 2008; Skrzynska et al., 2018). In freshwater medaka *Oryzias latipes*, salinity change also modulates nonapeptide levels (Haruta et al., 1991), while in rainbow trout, confinement increases VT expression (Gilchriest et al., 2000). Conversely, in zebrafish, VT levels do not change after acute or chronic unpredictable stress, despite increased cortisol (Pavlidis et al., 2015). Cortisol itself can modulate VT levels, as shown in sea bream (Cádiz et al., 2015). Manipulating the nonapeptide system can also affect stress physiology: VT administration can alter cortisol responses (Fryer and Leung, 1982), neurotransmitter levels (Gesto et al., 2014; Sangiao-Alvarellos et al., 2004), and ion regulation (Sangiao-Alvarellos et al., 2006). VT can modulate stress-related behaviours as well: for example, intracerebroventricular injection of VT in goldfish induced anorexigenic and anxiety-like behaviour (increased time spent at the bottom of the tank), which could be reversed with Manning compound (Araishi et al., 2019). The increase in the "fight" response by administration of the Manning compound observed here was unexpected, as VT is generally associated with increased aggression in fish, and VT antagonists usually decrease aggressiveness (e.g. Santangelo and Bass, 2006; Semsar et al., 2001; Yaeger et al., 2014). Notably, when the same pharmacological treatments were used in another experiment (Fusani et al., 2025), no effects were observed on aggressive behaviours. One speculative hypothesis is that VT receptor antagonist could act on pituitary corticotroph cells, which produce ACTH, and thereby modulate behaviour. This possibility is indirectly supported by three observations: 1) in vitro studies have shown that VT can act synergistically with CRH to stimulate ACTH release (Baker et al., 1996; Pierson et al., 1996); 2) the pituitary gland in fish is more permeable than the blood-brain barrier (Anbalagan et al., 2018), potentially allowing easier access to the pituitary itself, or possibly to central brain regions; 3) in zebrafish larvae, pituitary corticotroph cells have been shown to modulate locomotion, avoidance behaviours, and stimulus responsiveness after stress onset (De Marco et al., 2016). It should be noted, however, that in the study by De Marco et al. (2016), behavioural changes were accompanied by alterations in cortisol levels, whereas plasma cortisol did not change in the present study. Moreover, De Marco et al. (2016) currently provide the only direct evidence for such a pathway in fish, and extrapolating from zebrafish larvae to adult *B. splendens* is necessarily tentative given the substantial evolutionary divergence between these species (>100 million years, Zhang et al., 2022) and their different social systems. Future studies could directly assess whether the antagonist reaches the pituitary and/or central brain regions, and examine cellular activity markers, such as pRPS6 or equivalent indicators, in ACTH-producing cells and relevant neural circuits to clarify the mechanisms through which nonapeptide signalling modulates fight–flight–freeze responses in *B. splendens*. Finally, to confirm the involvement of VT in the fight–flight–freeze response, further research using VT receptor agonists (rather than only VT receptor antagonists) could contribute to further elucidate its precise action.

Despite the clear and visible behavioural and morphological (stripes) responses, plasma cortisol levels did not change, either at baseline or following administration of nonapeptide receptor antagonists. The assay has been previously validated in our laboratory and has reliably detected differences between groups in agonistic contexts (Ramos et al., 2021; Ramos and Gonçalves, 2022), suggesting that the null result reflects a genuine biological outcome rather than methodological limitations. To date, only one study (Cook et al., 2023) has measured cortisol levels after a looming test in fish, specifically in adult zebrafish, and found that whole-body cortisol was positively correlated with the number of looming disks presented. However, even though the correlation was significant, it was quite low ($R^2 = 0.173$). The relationship between defensive/antipredator behaviours and cortisol in fish remains unclear. For example, increases in cortisol have been observed in both juvenile winter flounder (Breves and Specker, 2005) and zebrafish (Barcellos et al., 2007) when exposed to predators, either through direct contact or purely visual cues. Similarly, in Nile tilapia (Sanches et al., 2015) elevated cortisol levels have been reported in response to conspecifics' alarm substances. At the same time, other studies have shown that clear antipredator behavioural responses are not always accompanied by changes in cortisol level, as seen in zebrafish (Barcellos et al., 2014), Nile tilapia (Miyai et al., 2016) and juvenile matrinxã *Brycon cephalus* (Ide et al., 2003). Additionally, schoolmaster snapper *Lutjanus apodus*, with elevated cortisol levels due to implants, did not differ in their behaviour from those with lower cortisol when subjected to predator-prey interactions (Lawrence et al., 2017). Thus, our results appear to align more closely with these latter studies, which suggest that cortisol may not be implicated in defensive behaviours in fish, in our case, in *B. splendens*. As it will be discussed in the context of colour change, catecholamines, rather than cortisol, may play a more significant role in modulating the stress response to the looming stimulus. Interestingly, to date, there are no studies specifically addressing the role of catecholamine in defensive/antipredator responses in fish.

Notably, this is the first study to quantitatively measure the appearance of stripes in fighting fish. Colour change can occur through either physiological or morphological mechanisms. The former is induced by acute, transient events, lasting from seconds to hours, resulting from the motility of pigment vesicles or reflective structures within the cells, caused by the redistribution of pre-existing structures (Alfakih et al., 2022; Leclercq et al., 2010). These structures, known as chromatophores (melanophores, erythrophores and xanthophores, and leucophores and iridophores), can be controlled by the nervous and endocrine system (Nery and Castrucci, 1997), with several pathways of action and targets involved (Nilsson Sköld et al., 2013). The latter mechanism of colour change is slower, occurring over days to weeks, and is associated with variations in skin pigment concentration and in the morphology, density, and distribution of chromatophores. This process involves pigment synthesis and degradation, and is typically linked to life-stage transitions (Alfakih et al., 2022; Leclercq et al., 2010).

Previous studies of stress-induced colour changes in fish, such as in the red porgy *Pagrus pagrus* (Van Der Salm et al., 2004a), the Atlantic mackerel *Scomber scombrus*, (Tveit et al., 2022), the cod *Gadus morhua* (Stien et al., 2005), and the Australian snapper *Pagrus auratus* (Doolan et al., 2009), have reported uniform body colouration changes in the context of aquaculture/welfare assessment. In contrast, disruptive colouration may be observed during anti-predator responses (Cuthill et al., 2005) due to the background pattern. However, the role of background in the expression of the disruptive pattern remains debated, as multiple factors likely influence prey detectability by predators (e.g., in humbug damselfish *Dascyllus aruanus* (Phillips et al., 2017), and least killifish *Heterandria formosa* (Kjernsmo and Merilaita, 2012)). Thus, the stripes observed here may be part of a defensive mechanism in *B. splendens*, but as existing reports are anecdotal and scientific literature is lacking, further research is warranted. In *B. splendens,* the stripes' appearance is also observed during mating, typically in females, and usually manifests as vertical stripes (Bronstein, 1982), though the underlying mechanisms and functional significance of this pattern remain unaddressed in published studies. To conclude, stripes were clearly associated with the looming response, but their significance and the ecological function across other contexts in *B. splendens* have yet to be investigated. Moreover, few studies have addressed chromatophore types in *B. splendens* (Khoo et al., 1997, 2012, 2014), leaving open questions about the mechanisms underlying stripe formation. However, regarding the

possible mechanisms controlling chromatophores and driving colour change, two options can be proposed: 1) hormonal action, specifically via α-MSH, or 2) a neuronal mechanism, possibly involving catecholamines. α-MSH is produced by cells in the *pars intermedia* of the pituitary. In vitro experiments using the pituitary gland of the red porgy, *P. pagrus*, showed that CRH, and thyrotropin-releasing hormone, stimulate α-MSH release, whereas dopamine and melanophore-concentrating hormone inhibit it (Van Der Salm et al., 2004b). MSHs stimulate not only melanophores but also other types of chromatophores, such as erythrophores, xanthophores, and leucophores, and different subtypes of receptors seem to be involved (Kobayashi et al., 2012). Under stress, α-MSH levels can increase, as seen in salmonids (Sumpter et al., 1985, 1986), in the gilthead sea bream *S. aurata* (Arends et al., 1999), and in the European sea bass *Dicentrarchus labrax* (Tsalafouta et al., 2017), although the relationship between α-MSH and stress-induced colour change remains poorly understood. Thus, measuring plasma α-MSH levels in response to threat stimuli could clarify whether this mechanism is involved. Regarding the second hypothesis, the role of catecholamines in mediating rapid colour change has been proposed in several species. For example, experiments have shown that incubation with norepinephrine (NE) induces chromatophore aggregation in the leopard coral trout *Plectropomus leopardus* (Zhao et al., 2022, 2024), Atlantic cod *G. morhua* (Aspengren et al., 2003), winter flounder *Pseudopleuronectes americanus* (Burton and Vokey, 2000), and freshwater snakehead *Channa punctatus* (Biswas et al., 2014). Another experiment in winter flounder demonstrated that incubation with high concentrations of NE induces melanosome aggregation, while low concentrations cause dispersion, reproducing the same pattern observed during stress (Burton, 2008). Given the rapid appearance of stripes in our study, occurring within seconds of the behavioural change, we hypothesise that the fast colour change observed during the looming stimulus in *B. splendens* is likewise mediated by a neuronal mechanism, probably involving catecholamines.

The animals used in this study can be considered relatively older than the usually tested *B. splendens*. Although in a previous study, fish of the same age showed a robust androgen response to a mirror challenge (Fusani et al., 2025), and the behavioural reaction to the looming stimulus was robust and expected, and no correlation with age was found for the hormonal response; one cannot exclude the possibility of an age-related effect. Hence, further research would be needed to confirm that the behavioural and endocrine response to the looming stimulus in this species, and the possible influence of the nonapeptides' system on it, is consistent across life stages.

5. Conclusion

In summary, we have, for the first time, characterised the response in *B. splendens* to a looming stimulus, demonstrating that the primary reaction is freezing, and that this behaviour is not associated with changes in cortisol levels. We also present a method to quantify stripe appearance, a key behavioural response to stressors in this species. Additionally, we show that blocking VT can alter the balance of fight–flight responses by decreasing flight and increasing fight behaviours. These results highlight the complexity of the defensive response in *B. splendens* and the potential role of nonapeptides in modulating these behaviours.

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CRediT authorship contribution statement

Bianca Fusani: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sara D. Cardoso:** Writing – review & editing, Software, Methodology, Investigation, Conceptualization. **Andreia Ramos:** Writing – review & editing, Methodology, Investigation, Conceptualization. **David Gonçalves:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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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References

Abramova, O., Zorkina, Y., Ushakova, V., Zubkov, E., Morozova, A., Chekhonin, V., 2020. The role of oxytocin and vasopressin dysfunction in cognitive impairment and mental disorders. Neuropeptides 83, 102079. https://doi.org/10.1016/J.NPEP.2020.102079.

Akinrinade, I., Kareklas, K., Teles, M.C., Reis, T.K., Gliksberg, M., Petri, G., Levkowitz, G., Oliveira, R.F., 2023. Evolutionarily conserved role of oxytocin in social fear contagion in zebrafish. Science (New York, N.Y.) 379 (6638), 1232–1237. https://doi.org/10.1126/SCIENCE.ABQ5158.

Alfakih, A., Watt, P.J., Nadeau, N.J., 2022. The physiological cost of colour change: evidence, implications and mitigations. J. Exp. Biol. 225 (10). https://doi.org/10.1242/jeb.210401.

Altmieme, Z., Jubouri, M., Touma, K., Coté, G., Fonseca, M., Julian, T., Mennigen, J.A., 2019. A reproductive role for the nonapeptides vasotocin and isotocin in male zebrafish (Danio rerio). Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 238, 110333. https://doi.org/10.1016/j.cbpb.2019.110333.

Alton, L.A., Portugal, S.J., White, C.R., 2012. Balancing the competing requirements of air-breathing and display behaviour during male-male interactions in Siamese fighting fish *Betta splendens*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 164 (2), 363–367. https://doi.org/10.1016/j.cbpa.2012.11.012.

Anbalagan, S., Gordon, L., Blechman, J., Matsuoka, R.L., Rajamannar, P., Wircer, E., Biran, J., Reuveny, A., Leshkowitz, D., Stainier, D.Y.R., Levkowitz, G., 2018. Pituicyte cues regulate the development of permeable neuro-vascular interfaces. Dev. Cell 47 (6), 711–726.e5. https://doi.org/10.1016/j.devcel.2018.10.017.

Guidelines for the treatment of animals in behavioural research and teaching. Anim. Behav. 83 (1), 2012, 301–309. https://doi.org/10.1016/j.anbehav.2011.10.031.

Araishi, K., Watanabe, K., Yamazaki, T., Nakamachi, T., Matsuda, K., 2019. Intracerebroventricular administration of arginine vasotocin (AVT) induces anorexigenesis and anxiety-like behavior in goldfish. Peptides 119, 170118. https://doi.org/10.1016/j.peptides.2019.170118.

Arends, R.J., Mancera, J.M., Muñ Oz, J.L., Bonga, W., Flik, G., 1999. The stress response of the gilthead sea bream (*Sparus aurata* L.) to air exposure and confinement. J. Endocrinol. 163, 149–157. https://doi.org/10.1677/joe.0.1630149.

Aspengren, S., Sköld, H.N., Quiroga, G., Mårtensson, L., Wallin, M., 2003. Noradrenaline- and melatonin-mediated regulation of pigment aggregation in fish melanophores. Pigment Cell Res. 16 (1), 59–64. https://doi.org/10.1034/j.1600-0749.2003.00003.x.

Baker, B.I., Bird, D.J., Buckingham, J.C., 1996. In the trout, CRH and AVT synergize to stimulate ACTH release. Regul. Pept. 67 (3), 207–210. https://doi.org/10.1016/S0167-0115(96)00130-9.

Ball, W., Tronick, E., 1971. Infant responses to impending collision: optical and real. Science 171 (3973), 818–820. https://doi.org/10.1126/science.171.3973.818.

Balment, R.J., Lu, W., Weybourne, E., Warne, J.M., 2006. Arginine vasotocin a key hormone in fish physiology and behaviour: A review with insights from mammalian models. Gen. Comp. Endocrinol. 147 (1), 9–16. https://doi.org/10.1016/j.ygcen.2005.12.022.

Barcellos, L.J.G., Ritter, F., Kreutz, L.C., Quevedo, R.M., da Silva, L.B., Bedin, A.C., Finco, J., Cericato, L., 2007. Whole-body cortisol increases after direct and visual contact with a predator in zebrafish, *Danio rerio*. Aquaculture 272 (1), 774–778. https://doi.org/10.1016/j.aquaculture.2007.09.002.

Barcellos, L.J.G., Koakoski, G., Da Rosa, J.G.S., Ferreira, D., Barreto, R.E., Giaquinto, P. C., Volpato, G.L., 2014. Chemical communication of predation risk in zebrafish does

not depend on cortisol increase. Sci. Rep. 4 (1), 5076. https://doi.org/10.1038/srep05076.
Batten, T.F.C., Moons, L., Vandesande, F., 1999. Innervation and control of the adenohypophysis by hypothalamic peptidergic neurons in teleost fishes: EM immunohistochemical evidence. Microsc. Res. Tech. 44 (1), 19–35. https://doi.org/10.1002/(SICI)1097-0029(19990101)44:1<19::AID-JEMT4>3.0.CO;2-L.
Bhattacharyya, K., McLean, D.L., MacIver, M.A., 2017. Visual threat assessment and reticulospinal encoding of calibrated responses in larval zebrafish. Curr. Biol. 27 (18), 2751–2762.e6. https://doi.org/10.1016/J.CUB.2017.08.012.
Biswas, S.P., Jadhao, A.G., Palande, N.V., 2014. Role of catecholamines and nitric oxide on pigment displacement of the chromatophores of freshwater snakehead teleost fish, *Channa punctatus*. Fish Physiol. Biochem. 40 (2), 457–467. https://doi.org/10.1007/s10695-013-9857-8.
Boccia, M.L., Goursaud, A.-P.S., Bachevalier, J., Anderson, K.D., Pedersen, C.A., 2007. Peripherally administered non-peptide oxytocin antagonist, L368,899®, accumulates in limbic brain areas: A new pharmacological tool for the study of social motivation in non-human primates. Horm. Behav. 52 (3), 344–351. https://doi.org/10.1016/j.yhbeh.2007.05.009.
Bower, T.G.R., Broughton, J.M., Moore, M.K., 1971. Infant responses to approaching objects: an indicator of response to distal variables. Percept. Psychophys. 9 (2), 193–196. https://doi.org/10.3758/BF03212627.
Braddock, J.C., Braddock, Z.I., 1955. Aggressive behavior among females of the Siamese fighting fish, *Betta splendens*. Physiol. Zool. 28, 152–172. https://doi.org/10.1086/PHYSZOOL.28.2.30163682.
Braida, D., Donzelli, A., Martucci, R., Capurro, V., Busnelli, M., Chini, B., Sala, M., 2012. Neurohypophyseal hormones manipulation modulate social and anxiety-related behavior in zebrafish. Psychopharmacology 220 (2), 319–330. https://doi.org/10.1007/s00213-011-2482-2.
Breves, J.P., Specker, J.L., 2005. Cortisol stress response of juvenile winter flounder (*Pseudopleuronectes americanus*, Walbaum) to predators. J. Exp. Mar. Biol. Ecol. 325 (1), 1–7. https://doi.org/10.1016/j.jembe.2005.04.019.
Bronstein, P.M., 1982. Breeding, paternal behavior, and their interruption in *Betta splendens*. Anim. Learn. Behav. 10 (2), 145–151. https://doi.org/10.3758/BF03212262.
Budaev, S.V., 2010. Using principal components and factor analysis in animal behaviour research: caveats and guidelines. Ethology 116 (5), 472–480. https://doi.org/10.1111/j.1439-0310.2010.01758.x.
Burton, D., 2008. A physiological interpretation of pattern changes in a flatfish. J. Fish Biol. 73 (3), 639–649. https://doi.org/10.1111/j.1095-8649.2008.01960.x.
Burton, D., Vokey, J.E., 2000. A1- and α2-adrenoceptor mediation in melanosome aggregation in cryptic patterning of *Pleuronectes americanus*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 125 (3), 359–365. https://doi.org/10.1016/S1095-6433(00)00166-5.
Cádiz, L., Román-Padilla, J., Gozdowska, M., Kulczykowska, E., Martínez-Rodríguez, G., Mancera, J.M., Martos-Sitcha, J.A., 2015. Cortisol modulates vasotocinergic and isotocinergic pathways in the gilthead sea bream. J. Exp. Biol. 218 (2), 316–325. https://doi.org/10.1242/jeb.113944.
Cannon, W.B., 1932. The Wisdom of the Body. W W Norton & Co.
Cook, A., Beckmann, H., Azap, R., Ryu, S., 2023. Acute stress modulates social approach and social maintenance in adult zebrafish. eNeuro 10 (9), ENEURO.0491-22.2023. https://doi.org/10.1523/ENEURO.0491-22.2023.
Cruz, A., Heinemans, M., Márquez, C., Moita, M.A., 2020. Freezing displayed by others is a learned Cue of danger resulting from co-experiencing own freezing and shock. Curr. Biol. 30 (6), 1128–1135.e6. https://doi.org/10.1016/J.CUB.2020.01.025.
Cuthill, I.C., Stevens, M., Sheppard, J., Haddocks, T., Párraga, C.A., Troscianko, T.S., 2005. Disruptive coloration and background pattern matching. Nature 434 (7029), 72–74. https://doi.org/10.1038/nature03312.
De Marco, R.J., Thiemann, T., Groneberg, A.H., Herget, U., Ryu, S., 2016. Optogenetically enhanced pituitary corticotroph cell activity post-stress onset causes rapid organizing effects on behaviour. Nat. Commun. 7 (1), 12620. https://doi.org/10.1038/ncomms12620.
Dill, L.M., 1974. The escape response of the zebra danio (*Brachydanio rerio*) I. The stimulus for escape. Anim. Behav. 22 (3), 711–722. https://doi.org/10.1016/S0003-3472(74)80022-9.
Domenici, P., 2002. The visually mediated escape response in fish: predicting prey responsiveness and the locomotor behaviour of predators and prey. Mar. Freshw. Behav. Physiol. 35 (1–2), 87–110. https://doi.org/10.1080/10236240290025635.
Doolan, B.J., Booth, M.A., Allan, G.L., Jones, P.L., 2009. Changes in skin colour and cortisol response of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801) to different background colours. Aquacult. Res. 40 (5), 542–550. https://doi.org/10.1111/j.1365-2109.2008.02126.x.
Døving, K.B., Hamdami, E.H., Höglund, E., Kasumyan, A.O., Tuvikene, A., 2005. A review on the chemical and physiological basis of alarm reactions in cyprinids. In: Reutter, K., Kapoor, B.G. (Eds.), Fish Chemosenses. Science Publishers, pp. 133–163.
Dunn, T.W., Gebhardt, C., Naumann, E.A., Riegler, C., Ahrens, M.B., Engert, F., Del Bene, F., 2016. Neural circuits underlying visually evoked escapes in larval zebrafish. Neuron 89 (3), 613–628. https://doi.org/10.1016/j.neuron.2015.12.021.
Fotowat, H., Gabbiani, F., 2007. Relationship between the phases of sensory and motor activity during a looming-evoked multistage escape behavior. J. Neurosci. 27 (37), 10047–10059. https://doi.org/10.1523/JNEUROSCI.1515-07.2007.
Friard, O., Gamba, M., 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods Ecol. Evol. 7 (11), 1325–1330. https://doi.org/10.1111/2041-210X.12584.
Fryer, J.N., Leung, E., 1982. Neurohypophysial hormonal control of cortisol secretion in the teleost *Carassius auratus*. Gen. Comp. Endocrinol. 48 (4), 425–431. https://doi.org/10.1016/0016-6480(82)90177-0.
Fusani, B., Ramos, A., Cardoso, S.D., Gonçalves, D., 2025. Vasotocin and oxytocin modulation of the endocrine and behavioral response to an aggressive challenge in male Siamese fighting fish. Horm. Behav. 171, 105728. https://doi.org/10.1016/j.yhbeh.2025.105728.
Gemmer, A., Mirkes, K., Anneser, L., Eilers, T., Kibat, C., Mathuru, A., Ryu, S., Schuman, E., 2022. Oxytocin receptors influence the development and maintenance of social behavior in zebrafish (*Danio rerio*). Sci. Rep. 12 (1), 4322. https://doi.org/10.1038/s41598-022-07990-y.
Gesto, M., Soengas, J.L., Rodríguez-Illamola, A., Míguez, J.M., 2014. Arginine Vasotocin treatment induces a stress response and exerts a potent anorexigenic effect in rainbow trout, *Oncorhynchus mykiss*. J. Neuroendocrinol. 26 (2), 89–99. https://doi.org/10.1111/jne.12126.
Gilchriest, B.J., Tipping, D.R., Hake, L., Levy, A., Baker, B.I., 2000. The effects of acute and chronic stresses on vasotocin gene transcripts in the brain of the rainbow trout (*Oncorhynchus mykiss*). J. Neuroendocrinol. 12 (8), 795–801. https://doi.org/10.1046/J.1365-2826.2000.00522.X.
Goncalves, D., Fusani, B., Cardoso, S.D., Canario, A.V.M., 2024. Hormones and sexual behavior of teleost fishes. In: Hormones and Reproduction of Vertebrates, *Volume 1: Fishes*, pp. 159–193. https://doi.org/10.1016/B978-0-443-16009-7.00006-2.
Gray, J.A., McNaughton, N., 2000. The Neuropsychology of Anxiety: An Enquiry into the Functions of the Septo-Hippocampal System. Oxford University Press.
Guillon, G., Derick, S., Pena, A., Cheng, L.L., Stoev, S., Seyer, R., Morgat, J.L., Barberis, C., Gal, C.S.-L., Wagnon, J., Manning, M., 2004. The discovery of novel vasopressin V1b receptor ligands for pharmacological, functional and structural investigations. J. Neuroendocrinol. 16 (4), 356–361. https://doi.org/10.1111/J.0953-8194.2004.01163.X.
Haruta, K., Yamashita, T., Kawashima, S., 1991. Changes in arginine vasotocin content in the pituitary of the medaka (*Oryzia latipes*) during osmotic stress. Gen. Comp. Endocrinol. 83 (3), 327–336. https://doi.org/10.1016/0016-6480(91)90137-U.
Hayes, W.N., Saiff, E.I., 1967. Visual alarm reactions in turtles. Anim. Behav. 15 (1), 102–106. https://doi.org/10.1016/S0003-3472(67)80018-6.
Holmqvist, M.H., 1994. A visually elicited escape response in the fly that does not use the giant fiber pathway. Vis. Neurosci. 11 (6), 1149–1161. https://doi.org/10.1017/S0952523800006957.
Ide, L.M., Urbinati, E.C., Hoffmann, A., 2003. The role of olfaction in the behavioural and physiological responses to conspecific skin extract in *Brycon cephalus*. J. Fish Biol. 63 (2), 332–343. https://doi.org/10.1046/j.1095-8649.2003.00152.x.
Jurek, B., Neumann, I.D., 2018. The oxytocin receptor: from intracellular signaling to behavior. Physiol. Rev. 98 (3), 1805–1908. https://doi.org/10.1152/physrev.00031.2017.
Kalueff, A.V., Gebhardt, M., Stewart, A.M., Cachat, J.M., Brimmer, M., Chawla, J.S., Craddock, C., Kyzar, E.J., Roth, A., Landsman, S., Gaikwad, S., Robinson, K., Baatrup, E., Tierney, K., Shamchuk, A., Norton, W., Miller, N., Nicolson, T., Braubach, O., Schneider, H., 2013. Towards a comprehensive Catalog of zebrafish behavior 1.0 and beyond. Zebrafish 10 (1), 70. https://doi.org/10.1089/ZEB.2012.0861.
Kareklas, K., Sorigue, P., Oliveira, R.F., 2025. Nonapeptide evolution and the regulation of social behaviour in teleost fish: from molecules to sociality, pp. 441–471. https://doi.org/10.1007/978-3-031-80209-6_15.
Kay, M., Elkin, L., Higgins, J.J., Wobbrock, J.O., 2025. *Mjskay/ARTool: ARTool 0.11.2* (Versione v0.11.2) [software]. Zenodo. https://doi.org/10.5281/ZENODO.594511.
Khoo, G., Loh, E.Y.F., Lim, T.M., Phang, V.P.E., 1997. Genetic variation in different varieties of Siamese fighting fish using isoelectric focusing of sarcoplasmic proteins. Aquac. Int. 5 (6), 537–549. https://doi.org/10.1023/A:1018357400571.
Khoo, G., Lim, T.M., Phang, V.P.E., 2012. Ultrastructure of erythrophores and xanthophores of the siamese fighting fish, *Betta splendens*. Isr. J. Aquacult. Bamidgeh 64. https://doi.org/10.46989/001C.20620.
Khoo, G., Lim, T.M., Phang, V.P.E., 2014. Cellular basis of metallic iridescence in the Siamese fighting fish, *Betta splendens*. http://hdl.handle.net/10524/49087.
King, J.G., Lettvin, J.Y., Gruberg, E.R., 1999. Selective, unilateral, reversible loss of behavioral responses to looming stimuli after injection of tetrodotoxin or cadmium chloride into the frog optic nerve. Brain Res. 841 (1–2), 20–26. https://doi.org/10.1016/S0006-8993(99)01764-3.
Kjernsmo, K., Merilaita, S., 2012. Background choice as an anti-predator strategy: the roles of background matching and visual complexity in the habitat choice of the least killifish. Proc. R. Soc. B Biol. Sci. 279 (1745), 4192–4198. https://doi.org/10.1098/rspb.2012.1547.
Kobayashi, Y., Mizusawa, K., Saito, Y., Takahashi, A., 2012. Melanocortin systems on pigment dispersion in fish chromatophores. Front. Endocrinol. 3, 9. https://doi.org/10.3389/fendo.2012.00009.
Kulczykowska, E., 2019. Arginine Vasotocin and Isotocin: towards their role in fish osmoregulation. In: Fish Osmoregulation, pp. 151–176. https://doi.org/10.1201/9780429063909-6.
Landin, J., Hovey, D., Xu, B., Lagman, D., Zettergren, A., Larhammar, D., Kettunen, P., Westberg, L., 2020. Oxytocin receptors regulate social preference in zebrafish. Sci. Rep. 10 (1), 1–12. https://doi.org/10.1038/s41598-020-61073-4.
Lawrence, M.J., Eliason, E.J., Brownscombe, J.W., Gilmour, K.M., Mandelman, J.W., Cooke, S.J., 2017. An experimental evaluation of the role of the stress axis in mediating predator-prey interactions in wild marine fish. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 207, 21–29. https://doi.org/10.1016/j.cbpa.2017.02.001.
Leclercq, E., Taylor, J.F., Migaud, H., 2010. Morphological skin colour changes in teleosts. Fish Fish. 11 (2), 159–193. https://doi.org/10.1111/j.1467-2979.2009.00346.x.
Ledoux, J., Daw, N.D., 2018. Surviving threats: neural circuit and computational implications of a new taxonomy of defensive behaviour. Nat. Rev. Neurosci. 19 (5), 269–282. https://doi.org/10.1038/nrn.2018.22.

Maciag, M., Doszyn, O., Wnorowski, A., Zmorzynska, J., Budzynska, B., 2025. Exploring the impact of MDMA and oxytocin ligands on anxiety and social responses: A comprehensive behavioural and molecular study in the zebrafish model. *Journal of Psychopharmacology* (Oxford, England) 39 (4), 373–393. https://doi.org/10.1177/02698811251324596.

Mahlmann, S., Meyerhof, W., Hausmann, H., Heierhorst, J., Schönrock, C., Zwiers, H., Lederis, K., Richter, D., 1994. Structure, function, and phylogeny of [Arg8]vasotocin receptors from teleost fish and toad. Proc. Natl. Acad. Sci. 91 (4), 1342–1345. https://doi.org/10.1073/PNAS.91.4.1342.

Mancera, J.M., Vargas-Chacoff, L., García-López, A., Kleszczyńska, A., Kalamarz, H., Martínez-Rodríguez, G., Kulczykowska, E., 2008. High density and food deprivation affect arginine vasotocin, isotocin and melatonin in gilthead sea bream (*Sparus auratus*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 149 (1), 92–97. https://doi.org/10.1016/j.cbpa.2007.10.016.

Manning, M., Misicka, A., Olma, A., Bankowski, K., Stoev, S., Chini, B., Durroux, T., Mouillac, B., Corbani, M., Guillon, G., 2012. Oxytocin and vasopressin agonists and antagonists as research tools and potential therapeutics. J. Neuroendocrinol. 24 (4), 609. https://doi.org/10.1111/J.1365-2826.2012.02303.X.

Martos-Sitcha, J.A., Wunderink, Y.S., Gozdowska, M., Kulczykowska, E., Mancera, J.M., Martínez-Rodríguez, G., 2013. Vasotocinergic and isotocinergic systems in the gilthead sea bream (*Sparus aurata*): an osmoregulatory story. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 166 (4), 571–581. https://doi.org/10.1016/J.CBPA.2013.09.001.

Martos-Sitcha, J.A., Fuentes, J., Mancera, J.M., Martínez-Rodríguez, G., 2014. Variations in the expression of vasotocin and isotocin receptor genes in the gilthead sea bream *Sparus aurata* during different osmotic challenges. Gen. Comp. Endocrinol. 197, 5–17. https://doi.org/10.1016/J.YGCEN.2013.11.026.

Maximino, C., Do Carmo Silva, R.X., Dos Santos Campos, K., De Oliveira, J.S., Rocha, S. P., Pyterson, M.P., Dos Santos Souza, D.P., Feitosa, L.M., Ikeda, S.R., Pimentel, A.F. N., Ramos, P.N.F., Costa, B.P.D., Herculano, A.M., Rosemberg, D.B., Siqueira-Silva, D.H., Lima-Maximino, M., 2019. Sensory ecology of ostariophysan alarm substances. J. Fish Biol. 95 (1), 274–286. https://doi.org/10.1111/jfb.13844.

Mbiydzenyuy, N.E., Qulu, L.A., 2024. Stress, hypothalamic-pituitary-adrenal axis, hypothalamic-pituitary-gonadal axis, and aggression. Metab. Brain Dis. 39 (8), 1613–1636. https://doi.org/10.1007/S11011-024-01393-W.

McKee, A., McHenry, M.J., 2020. The strategy of predator evasion in response to a visual looming stimulus in zebrafish (*Danio rerio*). Integrative Organismal Biology 2 (1). https://doi.org/10.1093/IOB/OBAA023.

Miyai, C.A., Sanches, F.H.C., Pinho-Neto, C.F., Barreto, R.E., 2016. Effects of predator odour on antipredator responses of Nile tilapia. Physiol. Behav. 165, 22–27. https://doi.org/10.1016/j.physbeh.2016.06.033.

Morton, F.B., Altschul, D., 2019. Data reduction analyses of animal behaviour: avoiding kaiser's criterion and adopting more robust automated methods. Anim. Behav. 149, 89–95. https://doi.org/10.1016/J.ANBEHAV.2019.01.003.

Nery, L.E.M., Castrucci, A.M.D.L., 1997. Pigment cell signalling for physiological color change. Comp. Biochem. Physiol. A Physiol. 118 (4), 1135–1144. https://doi.org/10.1016/S0300-9629(97)00045-5.

Nilsson Sköld, H., Aspengren, S., Wallin, M., 2013. Rapid color change in fish and amphibians—function, regulation, and emerging applications. Pigm. Cell Melanoma Res. 26 (1), 29–38. https://doi.org/10.1111/PCMR.12040.

Oliva, D., Tomsic, D., 2012. Visuo-motor transformations involved in the escape response to looming stimuli in the crab *Neohelice (=Chasmagnathus) granulata*. J. Exp. Biol. 215 (19), 3488–3500. https://doi.org/10.1242/jeb.070755.

Pavlidis, M., Theodoridi, A., Tsalafouta, A., 2015. Neuroendocrine regulation of the stress response in adult zebrafish, *Danio rerio*. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 60, 121–131. https://doi.org/10.1016/J.PNPBP.2015.02.014.

Phillips, G.A.C., How, M.J., Lange, J.E., Marshall, N.J., Cheney, K.L., 2017. Disruptive colouration in reef fish: does matching the background reduce predation risk? J. Exp. Biol. 220 (11), 1962–1974. https://doi.org/10.1242/JEB.151480.

Pierson, P.M., Guibbolini, M.E., Lahlou, B., 1996. A V1-type receptor for mediating the neurohypophysial hormone-induced ACTH release in trout pituitary. J. Endocrinol. 149 (1), 109–115. https://doi.org/10.1677/JOE.0.1490109.

Preuss, T., Osei-Bonsu, P.E., Weiss, S.A., Wang, C., Faber, D.S., 2006. Neural representation of object approach in a decision-making motor circuit. J. Neurosci. 26 (13), 3454–3464. https://doi.org/10.1523/JNEUROSCI.5259-05.2006.

R Core Team, 2021. R: A Language and Environment for Statistical Computing. https://www.R-project.org/.

Ramos, A., Gonçalves, D., 2019. Artificial selection for male winners in the Siamese fighting fish *Betta splendens* correlates with high female aggression. Front. Zool. 16, 34. https://doi.org/10.1186/s12983-019-0333-x.

Ramos, A., Gonçalves, D., 2022. Selection for winners impacts the endocrine system in the Siamese fighting fish. Gen. Comp. Endocrinol. 318, 113988. https://doi.org/10.1016/j.ygcen.2022.113988.

Ramos, A., Alex, D., Cardoso, S.D., Gonçalves, D., 2021. Androgens and corticosteroids increase in response to mirror images and interacting conspecifics in males of the Siamese fighting fish *Betta splendens*. Horm. Behav. 132, 104991. https://doi.org/10.1016/j.yhbeh.2021.104991.

Ramos, A., Gonçalves, D., Vasconcelos, R.O., 2025. Exploring freshwater soundscapes of tropical marshland habitats in Southeast Asia: insights into auditory sensory adaptation of wild Siamese fighting fish *Betta splendens*. PeerJ 13 (1), e18491. https://doi.org/10.7717/peerj.18491.

Rawat, A., Chaube, R., Joy, K.P., 2019. In situ localization of vasotocin receptor gene transcripts in the brain-pituitary-gonadal axis of the catfish *Heteropneustes fossilis*: A morpho-functional study. Fish Physiol. Biochem. 45 (3), 885–905. https://doi.org/10.1007/s10695-018-0590-1.

Ribeiro, D., Nunes, A.R., Gliksberg, M., Anbalagan, S., Levkowitz, G., Oliveira, R.F., 2020. Oxytocin receptor signalling modulates novelty recognition but not social preference in zebrafish. J. Neuroendocrinol. 32 (4). https://doi.org/10.1111/jne.12834.

Rivier, C., Vale, W., 1983. Modulation of stress-induced ACTH release by corticotropin-releasing factor, catecholamines and vasopressin. Nature 305 (5932), 325–327. https://doi.org/10.1038/305325A0.

Robertson, C.M., Sale, P.F., 1975. Sexual discrimination in the Siamese fighting fish (*Betta splendens* Regan). Behaviour 54 (1–2), 1–25. https://doi.org/10.1163/156853975X00308.

Sanches, F.H.C., Miyai, C.A., Pinho-Neto, C.F., Barreto, R.E., 2015. Stress responses to chemical alarm cues in Nile tilapia. Physiol. Behav. 149, 8–13. https://doi.org/10.1016/j.physbeh.2015.05.010.

Sangiao-Alvarellos, S., Lapido, M., Miguez, J.M., Soengas, J.L., 2004. Effects of central administration of arginine vasotocin on monoaminergic neurotransmitters and energy metabolism of rainbow trout brain. J. Fish Biol. 64 (5), 1313–1329. https://doi.org/10.1111/J.0022-1112.2004.00394.X.

Sangiao-Alvarellos, S., Polakof, S., Arjona, F.J., Kleszczynska, A., Martín del Río, M.P., Míguez, J.M., Soengas, J.L., Mancera, J.M., 2006. Osmoregulatory and metabolic changes in the gilthead sea bream *Sparus auratus* after arginine vasotocin (AVT) treatment. Gen. Comp. Endocrinol. 148 (3), 348–358. https://doi.org/10.1016/J.YGCEN.2006.04.005.

Santangelo, N., Bass, A.H., 2006. New insights into neuropeptide modulation of aggression: field studies of arginine vasotocin in a territorial tropical damselfish. Proceedings. Biological sciences 273 (1605), 3085–3092. https://doi.org/10.1098/rspb.2006.3683.

Satao, K.S., Doshi, G.M., 2024. Anxiety and the brain: neuropeptides as emerging factors. Pharmacol. Biochem. Behav. 245, 173878. https://doi.org/10.1016/J.PBB.2024.173878.

Schiff, W., Caviness, J.A., Gibson, J.J., 1962. Persistent fear responses in rhesus monkeys to the optical stimulus of «looming». Science 136 (3520), 982–983. https://doi.org/10.1126/SCIENCE.136.3520.982.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9 (7), 676–682. https://doi.org/10.1038/nmeth.2019.

Semsar, K., Godwin, J., 2004. Multiple mechanisms of phenotype development in the bluehead wrasse. Horm. Behav. 45 (5), 345–353. https://doi.org/10.1016/j.yhbeh.2004.01.003.

Semsar, K., Kandel, F.L.M., Godwin, J., 2001. Manipulations of the AVT system shift social status and related courtship and aggressive behavior in the bluehead wrasse. Horm. Behav. 40 (1), 21–31. https://doi.org/10.1006/hbeh.2001.1663.

Skrzynska, A.K., Gozdowska, M., Kulczykowska, E., Martínez-Rodríguez, G., Mancera, J. M., Martos-Sitcha, J.A., 2017. The effect of starvation and re-feeding on vasotocinergic and isotocinergic pathways in immature gilthead sea bream (*Sparus aurata*). Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 187 (7), 945–958. https://doi.org/10.1007/S00360-017-1064-Y.

Skrzynska, A.K., Maiorano, E., Bastaroli, M., Naderi, F., Míguez, J.M., Martínez-Rodríguez, G., Mancera, J.M., Martos-Sitcha, J.A., 2018. Impact of air exposure on vasotocinergic and isotocinergic systems in gilthead sea bream (*Sparus aurata*): new insights on fish stress response. Front. Physiol. 9, 96. https://doi.org/10.3389/fphys.2018.00096.

Speedie, N., Gerlai, R., 2008. Alarm substance induced behavioral responses in zebrafish (*Danio rerio*). Behav. Brain Res. 188 (1), 168–177. https://doi.org/10.1016/j.bbr.2007.10.031.

Stien, L.H., Hirmas, E., Bjørnevik, M., Karlsen, Ø., Nortvedt, R., Rørå, A.M.B., Sunde, J., Kiessling, A., 2005. The effects of stress and storage temperature on the colour and texture of pre-rigor filleted farmed cod (*Gadus morhua* L.). Aquacult. Res. 36 (12), 1197–1206. https://doi.org/10.1111/j.1365-2109.2005.01339.x.

Sumpter, J.P., Pickering, A.D., Pottinger, T.G., 1985. Stress-induced elevation of plasma α-MSH and endorphin in brown trout, *Salmo trutta* L. Gen. Comp. Endocrinol. 59 (2), 257–265. https://doi.org/10.1016/0016-6480(85)90377-6.

Sumpter, J.P., Dye, H.M., Benfey, T.J., 1986. The effects of stress on plasma ACTH, α-MSH, and cortisol levels in salmonid fishes. Gen. Comp. Endocrinol. 62 (3), 377–385. https://doi.org/10.1016/0016-6480(86)90047-X.

Sun, H., Frost, B.J., 1998. Computation of different optical variables of looming objects in pigeon nucleus rotundus neurons. Nat. Neurosci. 1 (4), 296–303. https://doi.org/10.1038/1110.

Temizer, I., Donovan, J.C., Baier, H., Semmelhack, J.L., 2015. A visual pathway for looming-evoked escape in larval zebrafish. Curr. Biol. 25 (14), 1823–1834. https://doi.org/10.1016/J.CUB.2015.06.002.

Theofanopoulou, C., Gedman, G., Cahill, J.A., Boeckx, C., Jarvis, E.D., 2021. Universal nomenclature for oxytocin-vasotocin ligand and receptor families. Nature 592. https://doi.org/10.1038/s41586-020-03040-7.

Tronick, E., 1967. Approach response of domestic chicks to an optical display. J. Comp. Physiol. Psychol. 64 (3), 529–531. https://doi.org/10.1037/h0025206.

Tsalafouta, A., Gorissen, M., Pelgrim, T.N.M., Papandroulakis, N., Flik, G., Pavlidis, M., 2017. α-MSH and melanocortin receptors at early ontogeny in European sea bass (*Dicentrarchus labrax*, L.). Scientific reports 2017 7:1, 7 (1), 1–9. https://doi.org/10.1038/srep46075.

Tseng, Y.T., Schaefke, B., Wei, P., Wang, L., 2023. Defensive responses: behaviour, the brain and the body. Nature reviews neuroscience 2023 24:11, 24 (11), 655–671. https://doi.org/10.1038/s41583-023-00736-3.

Tveit, G.M., Anders, N., Bondø, M.S., Mathiassen, J.R., Breen, M., 2022. Atlantic mackerel (*Scomber scombrus*) change skin colour in response to crowding stress. J. Fish Biol. 100 (3), 738–747. https://doi.org/10.1111/JFB.14987.

Uvnäs-Moberg, K., Gross, M.M., Calleja-Agius, J., Turner, J.D., Vaudry, H., Sona, C., Xiao, L., 2024. The yin and Yang of the oxytocin and stress systems: opposites, yet interdependent and intertwined determinants of lifelong health trajectories. Front. Endocrinol. 15, 1272270. https://doi.org/10.3389/FENDO.2024.1272270.

Van Der Salm, A.L., Martínez, M., Flik, G., Wendelaar Bonga, S.E., 2004a. Effects of husbandry conditions on the skin colour and stress response of red porgy, *Pagrus pagrus*. Aquaculture 241 (1–4), 371–386. https://doi.org/10.1016/J.AQUACULTURE.2004.08.038.

Van Der Salm, A.L., Pavlidis, M., Flik, G., Wendelaar Bonga, S.E., 2004b. Differential release of α-melanophore stimulating hormone isoforms by the pituitary gland of red porgy, *Pagrus pagrus*. Gen. Comp. Endocrinol. 135 (1), 126–133. https://doi.org/10.1016/J.YGCEN.2003.09.002.

Williams, A.V., Duque-Wilckens, N., Ramos-Maciel, S., Campi, K.L., Bhela, S.K., Xu, C.K., Jackson, K., Chini, B., Pesavento, P.A., Trainor, B.C., 2020. Social approach and social vigilance are differentially regulated by oxytocin receptors in the nucleus accumbens. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 45 (9), 1423–1430. https://doi.org/10.1038/s41386-020-0657-4.

Wilson, L.C., Riordan, A., Nussbaum, A., Krawitz, J., 2024. Heart and shoal: social cues and oxytocin receptors impact stress recovery in the zebrafish. Physiol. Behav. 283, 114613. https://doi.org/10.1016/J.PHYSBEH.2024.114613.

Yaeger, C., Ros, A.M., Cross, V., DeAngelis, R.S., Stobaugh, D.J., Rhodes, J.S., 2014. Blockade of arginine vasotocin signaling reduces aggressive behavior and c-Fos expression in the preoptic area and periventricular nucleus of the posterior tuberculum in male *Amphiprion ocellaris*. Neuroscience 267, 205–218. https://doi.org/10.1016/j.neuroscience.2014.02.045.

Yang, W., Zhang, N., Wu, Y., Zhang, L., Zhang, L., Zhang, W., 2021. Oxytocin-like signal regulates Lh cells directly but not Fsh cells in the ricefield eel *Monopterus albus*. Biol. Reprod. 104 (2), 399–409. https://doi.org/10.1093/BIOLRE/IOAA202.

Yilmaz, M., Meister, M., 2013. Rapid innate defensive responses of mice to looming visual stimuli. Curr. Biol. 23 (20), 2011–2015. https://doi.org/10.1016/J.CUB.2013.08.015.

Zacarias, R., Namiki, S., Card, G.M., Vasconcelos, M.L., Moita, M.A., 2018. Speed dependent descending control of freezing behavior in *Drosophila melanogaster*. Nat. Commun. 9, 3697 (2018). https://doi.org/10.1038/s41467-018-05875-1.

Zhang, W., Wang, H., Brandt, D.Y.C., Hu, B., Sheng, J., Wang, M., Luo, H., Li, Y., Guo, S., Sheng, B., Zeng, Q., Peng, K., Zhao, D., Jian, S., Wu, D., Wang, J., Zhao, G., Ren, J., Shi, W., Hong, Y., 2022. The genetic architecture of phenotypic diversity in the Betta fish (*Betta splendens*). Sci. Adv. 8 (38), eabm4955. https://doi.org/10.1126/sciadv.abm4955.

Zhao, N., Ge, X., Jiang, K., Huang, J., Wei, K., Sun, C., Xi Chen, S., Mizusawa, K., Pratap Singh, A., Sköld, H., 2022. Ultrastructure and regulation of color change in blue spots of leopard coral trout *Plectropomus leopardus*. Front. Endocrinol. 13, 984081. https://doi.org/10.3389/FENDO.2022.984081.

Zhao, N., Jiang, K., Ge, X., Huang, J., Wu, C., Chen, S.X., 2024. Neurotransmitter norepinephrine regulates chromatosomes aggregation and the formation of blotches in coral trout *Plectropomus leopardus*. Fish Physiol. Biochem. 50 (2), 705–719. https://doi.org/10.1007/s10695-024-01300-1.