Comparing behavioral performance and physiological responses of *Sebastes schlegelii* with different aggressive strengths

Haixia Li
Dalian Ocean University
Jie Wang
Dalian Ocean University
Xu Zhang
Dalian Ocean University
Yu Hu
Dalian Ocean University
Ying Liu
Zhejiang University School of Biosystems Engineering and Food Science
Zhen Ma (mazhen@dlou.edu.cn)
Dalian Ocean University https://orcid.org/0000-0001-9859-9637

Research Article

Keywords: aggressive strengths, behavioral performance, cortisol, monoamines, Sebastes schlegelii

Posted Date: February 16th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1345435/v1

Abstract

Fish aggression has significant individual differences, and different personalities exhibit distinct behavioral performances and physiological stress responses. Under intensive culture conditions, *Sebastes schlegelii* juveniles display severe aggression and cannibalism, causing fish welfare and economic loss. Herein, we investigated the alterations in behavioral performance and physiological stress indicators of *Sebastes schlegelii* juveniles with different aggressive strengths. The results revealed that latency to the first movement, distance to center-point, mobile frequency, and immobile frequency were significantly lower in high-aggressive individuals than low-aggressive individuals. In contrast, the immobile time was significantly higher in high-aggressive individuals compared to low-aggressive individuals. PCA was performed to identify the key parameters of fish behavior. From the results of PCA, position, motion state, and physical status could be used as behavioral screening indicators for individuals with different aggressions. The 5-HIAA/5-HT ratio was significantly lower in high-aggressive individuals than in low-aggressive individuals. Moreover, cortisol levels were positively correlated with immobile time, and the ratio of 5-HIAA/5-HT was significantly and positively correlated with the distance to the central point. These results suggest that different aggressive individuals can be identified according to position, motion state, and physical status in a short period of time, while the distance to center-point associated with brain monoaminergic activity may be a more accurate indicator. The results not only provide a non-invasive method for reducing fish aggression but also help to enhance precise management in aquaculture.

1. Introduction

Sebastes schlegelii, a carnivorous fish species commonly known as black rockfish, is one of the vital economic fish species in the coastal areas of the Yellow Sea and Bohai Sea of China. Due to overfishing, environmental damage, and habitat degradation, black rockfish resources have decreased sharply in recent years (Lü et al. 2014; Xi et al. 2017a). As a regional fish species with tremendous development potential, black rockfish aquaculture has developed rapidly in recent years. In general, black rockfish were continuously cultured to over 12 cm under controlled conditions before being released in the coastal seas or moved into nets and were used as a food source and to relieve fishing pressure (Guo et al. 2017; Xu et al. 2020). The aim of aquaculture is to improve the survival rate of juveniles; however, under intensive culture conditions, black rockfish juveniles display serious aggression and cannibalism, leading to fish welfare and economic loss (Xi et al. 2017b; Xu et al. 2020).

The fundamental definition of fish welfare includes a state of complete physical and normal behavior (Rault et al. 2020). Under natural conditions, aggression is an adaptive behavior intended to protect one from harm and conserve resources. However, in intensive aquaculture, cultured fishes may have little or no access to escape predation (Metcalfe et al. 2016; Xu et al. 2020). The most direct impact of aggressive behavior is physical impairment, which in turn influences the later health and motility of fish (Brandão et al. 2018; Castanheira et al. 2013; Wolkers et al. 2017). Fish aggression influences the effectiveness and sustainability of aquaculture, such as growth, physical condition, welfare, and food conversion. As a result, various methods should be used to reduce aggression and cannibalism, regardless of the fish being farmed (Hvas et al. 2021; Manuel et al. 2016).

The occurrence and degree of aggressive behavior vary widely among fishes. There are surprising and consistent differences between the fighting parties, even among individuals of the same population, gender, size, and age (Hellinger et al. 2020; Hu et al. 2021; Huntingford and Adams 2005; Zabegalov et al. 2019). In other words, some fish fight more than others. This is not noise but an essential biological variability that must be recognized and rationalized. Although fish personalities are distinct, this does not mean that the potential underlying laws of behavioral expression cannot be elucidated. Fish aggression is complex and includes not only direct biting and nipping but also differing displays and postures (Haraldstad et al. 2019; Qian et al. 2017). With the increased intensity of behavioral expression, the extensive patterns that eventually lead to bite attacks are typical, allowing the possibility of uncovering a pattern in fish behavior.

Fish behavior is a comprehensive representation of extrinsic environmental factors and intrinsic physiological factors (Xu et al. 2020). There have been multiple studies on the physiological research of aggressive fish behavior (Ariyomo et al. 2013; Boscolo et al. 2018), but studies about behavioral performance are scarce. In this paper, we aimed to distinguish the main characteristics of juveniles with contrasting aggressive patterns to identify the features and patterns of aggressive behavior in black rockfish. The results will not only provide a viable and non-invasive method for reducing fish aggression and improving fish welfare but also enhance management in aquaculture, such as optimizing culture systems according to the behavioral performance, purposefully screening specific objects, and domesticating specific behavioral groups.

2. Materials And Methods

We have read the policies relating to animal experiments and confirmed this study complied (ARRIVE guidelines; EU Directive 2010/63/EU for animal experiments). All procedures performed in this study involving animals were in accordance with the guidelines and ethical standards of the Dalian Ocean University.

2.1. Source and experimental animal

Experiments were conducted at Key Laboratory of Environment Controlled Aquaculture (AET), Dalian city, Liaoning Province, China, 2020. Black rockfish juveniles with the same genetic background and from the same population were obtained from the laboratory and temporarily reared and acclimated for 14 days. Juveniles were fed commercial feed (crude protein ≥ 44%, crude fat ≥ 5%) at the rate of 2% of their body mass 3 times daily. The water temperature was maintained at 20 ± 1°C, and natural illumination was used throughout the study. Fish were fasted on the day prior to the experiment.

2.2. Experimental design

Dyadic fighting (DF) was used to stimulate aggressive interactions in this experiment (Oliveira et al. 2011). To exclude the influence of body size on aggression, fish specifications were strictly screened according to the following criteria: body length and weight variations were less than 0.1 cm and less than 5%, respectively. A total of 71 juveniles were used with an initial body length of 6.50 ± 0.10 cm and body weight of 7.70 ± 0.30 g. Ten glass tanks of the same size (21×14×17.5 cm) were used herein. The back and lateral sides of the tank were covered with nontransparent white plastic boards to increase contrast and reduce external visual clues during behavioral recording. Cameras (HIK-DS-2CD3T35D-I5) were directly mounted above and in front of the tanks.

Two randomly selected juveniles were placed in the experimental tank and separated by a removable divider. Two days later, the divider was removed, and aggressive interactions were videotaped and registered by recording the time of occurrence. Each video lasted 10 min. For each aggressive interaction, the initiator, the receiver, the winner, the loser as well as the duration of the fight were documented. Winner/loser outcomes could be easily identified and determined by different behavioral performances (Teles and Oliveira 2016). The winner was defined as high aggression (H-agg), and the loser was defined as low aggression (L-agg). The same procedure was performed for the control group (N = 9) but without divider removal.

2.3. Behavioral observations

The activity of the juveniles was tracked and calculated using Noldus EthoVision XT (version 12.0; Noldus Information Technology, Netherlands). A dynamic subtraction method was applied, and the sampling rate was set at 25 frames per second. A smoothing (Lowess) method was used to eliminate small movements such as system background noise. The tracking data were additionally checked by an all-occurrence recording method to ensure the accuracy of the results.

The initiator of the aggressive behavior was defined as the target fish, and the other was defined as the object fish. The frequency and duration of the occurrence of aggressive behavior were recorded, respectively. To analyze behavioral differences in individuals with different aggressive strengths, behavioral parameters related to aggression were further quantified, divided into 3 aspects: position, motion state, and physical status. Ten parameters related to aggressive behavior were determined: distance moved (cm), velocity (cm/s), acceleration (cm/s^2), angular velocity (º/s), latency to the first movement (s), distance to center-point (cm), mobile time (s), mobile frequency, immobile time (s) and immobile frequency. Lastly, the principal component analysis (PCA) was used to identify variations in behavioral parameters of the fish aggression process.

2.4. Sampling and analytical methods

At the end of the trial, all juveniles in control group and same number juveniles in treatment group were anesthetized, and the tissues were sampled. The brain tissues of each juvenile were sampled with clean surgical scissors and pointed tweezers to avoid contamination. Afterward, the tissues were flash-frozen in liquid nitrogen and stored at − 80°C. Lastly, the activity of 5-hydroxytryptamine (5-HT), 5-

hydroxyindoleacetic acid (5-HIAA), and cortisol were analyzed by double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kits (Meimian, Jiangsu, China).

2.5. Data analysis

All values were presented as means ± standard error of the mean (SEM). Statistical analysis was performed using Microsoft Office Excel 2017and SPSS 21.0. $P \leq 0.05$ was considered to be statistically significant. Nonparametric tests were used when data were not normally distributed. Correlations were examined by the Spearman rank correlation test.

3. Results

3.1. Behavioral performance

The results obtained from the behavioral analysis of individuals with different aggression are presented in Fig. 1. Both the latency to the first movement and the distance to the center point were significantly higher in the low-aggression group than those in the high-aggression group. The angular velocity was lower in individuals with high aggression than that of low-aggression individuals, but no significant differences were observed. Heat maps of dwell time demonstrated the selection of arena by individuals with different aggression. It appeared that, regardless of movement and locomotion quiescence, individuals with high aggression were mostly situated in the central area of the experimental system. On the contrary, individuals with low aggression were mostly located at the system's edge. Aside from that, the results also determined that high aggression individuals were mostly in a dynamic state, while low aggression individuals were mostly stationary.

The motion state of the high-aggression group did not differ significantly from that of the low-aggression group ($P > 0.05$; Fig. 3). The high-aggression group had lower movement velocity, lower total distance moved, and higher acceleration, but not significantly different than those in the low-aggression group ($P >$ 0.05; Fig. 3). The acceleration was significantly higher in the high-aggression group than that in the low-aggression group ($P \leq 0.05$; Fig. 3). The physical status of the high-aggression group was significantly different from that of the low-aggression group ($P \leq 0.05$; Fig. 4). Likewise, there was no difference in mobile time between the high-aggression and low-aggression groups, but there is a noticeable difference in mobile frequency. Indeed, the mobile frequency was significantly higher in the low-aggression group than in the high-aggression group. The immobile time in individuals with high aggression was markedly longer than those with low aggression, but the immobile frequency was significantly lower than that of low-aggression individuals ($P \leq 0.05$; Fig. 4).

Figure 5 presents the frequency and duration of behavior of highly aggressive individuals within 10 min to characterize the physical interaction. The results illustrated that the trends of aggression duration and frequency were generally consistent, showing a gradual decrease in duration and frequency over time, with a progressive leveling of aggression frequency and duration after 300 s (Fig. 5).

3.2. Key behavioral parameters extraction

The results of the key behavioral parameters extraction for individuals with different aggressive strengths are outlined in Table 1. For aggressive behavior, the first variable factor (CF1) accounted for 22.80% of the total variation and had a strong positive loading on velocity (0.831). The analysis revealed that the first component of the PCA mainly reflected the movement state of high and low aggression individuals. The second variable factor (VF2) accounted for 20.09% of the total variance, and the immobile time had a strong negative loading (-0.851), while the immobile frequency had a strong positive loading (0.859). VF2 could be interpreted as the physical state of high and low aggressive individuals. The third variable factor (VF3) accounted for 18.92% of the total variance and had a strong positive loading on the distance to center-point (0.805), interpreted as the location distribution of individuals with contrasting aggressions.

Table 1
PCA results of aggressive behavior in high and low aggressive individuals

Variables	**VF1**	**VF2**	**VF3**
Behavioral performance of aggressivity(three significant components)			
Latency to first move	-0.682	-0.273	0.394
Distance to center-point	-0.031	0.243	**0.805**
Angular velocity	0.455	0.345	0.130
Velocity	**0.831**	-0.287	0.201
Acceleration	-0.077	0.070	-0.523
Mobile time	0.307	-0.077	0.638
Mobile frequency	0.764	-0.014	0.355
Immobile time	0.034	**-0.851**	-0.123
Immobile frequency	0.043	**0.859**	-0.144
Rotation sums of squared loadings			
Total	2.052	1.808	1.702
% Total variance	22.803	20.094	18.915
Cumulative % variance	22.803	42.898	61.813

3.3. Cortisol levels and brain monoaminergic activity

The results showed that the cortisol levels were significantly higher in both high and low aggressive individuals compared to the control group ($F_{2,24}$ = 16.14, $P \leq 0.01$), but there was no significant difference in cortisol levels between high-aggressive and low-aggressive individuals ($P > 0.05$, Fig. 6). The 5-HT of individuals with high and low aggression was significantly higher than those in the control group

($F_{2,24}$ = 18.16, $P \leq 0.01$). Similarly, there was no significant difference in 5-HT levels between individuals with different aggression ($P > 0.05$, Fig. 7). The 5-HIAA/5-HT ratio was significantly higher in individuals with low aggression than that in individuals with high aggression ($F_{2,24}$ = 7.60, $P \leq 0.01$). Again, there was no significant difference in 5-HIAA/5-HT ratio between the control and the other two groups ($P > 0.05$, Fig. 8).

3.4. Correlation of physiological indicators with behavioral analysis

The relationship between the key behavioral parameters extracted by PCA and cortisol responses is depicted in Fig. 9. Cortisol levels were positively correlated with immobile time ($P \leq 0.05$, Fig. 9). The relationship between the key behavioral parameters extracted by PCA and the 5-HIAA/5-HT ratio is delineated in Fig. 10. Furthermore, the ratio of 5-HIAA/5-HT was significantly and positively correlated with the distance to the central point ($P \leq 0.05$, Fig. 10).

4. Discussion

4.1. Behavioral performance

At the present stage, the study of the aggressiveness of fish mainly adopts mirror image stimulation tests (Falsarella et al. 2017; Gerlai et al. 2000; Liu et al. 2020; Marks et al. 2005; Moretz et al. 2007a; Moretz et al. 2007b) and real opponent stimulation tests (Aguiar and Giaquinto 2018; Boscolo et al. 2018; Brandão et al. 2018; Fontana et al. 2016; Zhang et al. 2020; Zhang et al. 2021). The mirror test is a standardized experimental method that can stimulate the same aggressive behavior in the opponent fish and the target fish. However, some studies have pointed out that hormone levels, behavioral responses, and physiological changes caused by the aggressive response stimulated by the mirror test are different from those caused by real opponent stimulation (Hirschenhauser et al. 2008; Oliveira et al. 2016). Compared with the mirror image test, the real opponent stimulation test induces a more complete expression of aggressive behavior in the opponent fish and the target fish, and the behavior sequence is more complex, which can trigger a behavioral response that is closest to reality and form a typical aggressive phenotypic difference individuals (Hubená et al. 2020). Some studies reported that the real opponent stimulation test could accurately divide aggressiveness after about 5 min (Ariyomo et al. 2013; Teles and Oliveira 2016). Therefore, it is more realistic and accurate to adopt stimulating experiments with real opponents (Teles and Oliveira 2016).

Three related behavioral factors were selected from the 10 parameters related to aggressive behavior, namely, the individual's position, motion state, and physical status. All three factors can represent the significant differences between high aggression and low aggression individuals and can also be used as typical behavioral parameters for rapidly screening individuals with diverse aggression (Colléter and Brown 2011; Oliveira et al. 2011; Quadros et al. 2018). From the perspective of individual motion states, there was no significant difference between the attack speed of high aggression and escape speed of low aggression, which may be due to the small experimental area, leaving limited space for individuals to attack or escape (Ma et al. 2021). The distribution position results determined that aggression was significantly associated with fish personality. Individuals with high aggression usually adopt active coping strategies (approach-attack), while individuals with low aggression usually display passive coping strategies (defense-escape) (Oliveira et al. 2011). The results revealed that the latency to the first movement of high aggression individuals is shorter and closer to the center of the experimental system. In contrast, the latency to the first movement of low aggression individuals is longer, closer to the edge of the experimental system, and with a longer residence time. This finding is consistent with that of Colléter (Colléter and Brown 2011). Assuming that there is no shelter, the moving tendency and position in an open water tank can represent the individual's boldness (Dahlbom et al. 2011). Individuals with a strong tendency to move and who are often located in the central area are typically considered bold ones, while individuals with a weak mobility tendency and often located in the marginal area are considered shy (Oliveira et al. 2011). Therefore, individuals with high aggression are often bolder than individuals with low aggression. In terms of individual physical state, it is interesting to note that the activity of individuals with high aggression is lower than individuals with low aggression. This finding is contrary to most previous studies (Quadros et al. 2018; Yuan et al. 2018). The reasons may be as follows: First, the method used to measure fish activity in this study differs from the general approach, which is measured separately according to the area that fish passed through per unit time. The activity in this experiment was measured in real-time according to the percentage change in the number of pixels when the object moved (Hubená et al. 2020; Whittaker et al. 2021). Obviously, the change in measurement, based on the moving object itself, is more reasonable and accurate. Secondly, during short-term interactions, individuals with low aggression may be more inclined to defend and retreat due to limited space for hiding or escaping (Ma et al. 2021; Paull et al. 2010). This results in more struggling actions, such as swinging the tail in the edge area of the test system, leading to a higher rate in pixel change. Thirdly, studies have established that individuals with high aggression are less flexible to daily changes than individuals with low aggression (Vindas et al. 2017a), suggesting that individuals with high aggression may be less active.

Aggressive behavior can be divided into proactive and reactive aggression based on specific behavioral and physiological characteristics (Wrangham 2018; Zhu et al. 2019). Proactive aggression is triggered by scheduled motivations to achieve personal goals or obtain personal gains (Fanning et al. 2020; Ibabe 2020). Therefore, proactive aggression is often scheduled premeditated, and is associated with lower emotional responsiveness. Conversely, reactive aggression is driven by provocation and/or perceived threat and is usually impulsive and unplanned. Moreover, it is usually linked to higher emotional responsiveness (Dorfman et al. 2013; Fanning et al. 2020; Zhu et al. 2019). The present results showed that the high-frequency aggression in individuals with high aggression was manifested mostly as time concentration, short persistent time, and high repeatability. This result is impressive, as it indicates that the aggressive behavior of individuals with high aggression may actually belong to reactive aggression (Cervantes and Delville 2007).

Herein, differences between different types of aggression were not taken into account. The rationale is that the differences are visible by the naked eye, regardless of aggression frequency and behavioral performance. Individuals with low aggression showed obvious escape and freezing behavior, while high aggression individuals mainly exhibited biting and chasing behavior (Oliveira et al. 2011). In addition, aggression tests that involve three or more interacting individuals were not addressed in the real opponent stimulation test. Earlier studies indicated that different experimental methods lead to differences in behavioral performances between high and low aggression individuals (Oliveira et al. 2005; Way et al. 2015). In fact, fishes form a specific and self-organized structure during group formation. Therefore, the aggressive behavior of fish in a group context is more reflecting of the real aggression situation (Xu et al. 2020). Regrettably, this is challenging to achieve in practice at this stage.

4.2 Physiological indicators

Variations in cortisol levels have been widely used as a stress indicator in fishes (Moltesen et al. 2016). The cortisol responses suggested that the individuals exposed to an aggressive state (high and low) were under significant stress (higher cortisol levels at baseline), which was in agreement with Bessa's finding (Bessa et al. 2021). It is somewhat surprising that no significant difference in cortisol was found between the two aggressive groups. This indicated that the cortical responses evoked by short-duration aggression were similar, regardless of individuals with high or low aggression. This finding does not support the previous research (Sherman and Mehta 2019; Sloman et al. 2001). Studies have shown that the decrease in cortisol levels after stress is more important than the cortisol concentration during stress (Koolhaas et al. 2011), that is, individuals with different aggression may have similar cortisol concentrations at the beginning of the aggression. Another possible reason might be that cortisol was persistently elevated after 10 minutes of aggression (Moltesen et al. 2016).

In teleost fishes, differences in brain monoamine activity have been consistently and reliably utilized to reflect the motivation and outcome of fish aggression (Backström and Winberg 2017; Clotfelter et al. 2007). Considering that monoamines and their metabolites require a certain period of time to accumulate, the 5-HIAA/5-HT ratio may be a more reliable indicator of neural activity for a short duration (Øverli et al. 1999). This can be used to justify the lack of significant difference in the 5-HT content, but the 5-HIAA/5-HT ratio was significantly different in individuals with various aggressive behavior. This finding was also reported by Loveland et al. (Loveland et al. 2014). During aggression, the brain's 5-HT-ergic system was activated, and the activity of 5-HT increased rapidly in low aggression individuals.

In this study, there is a decent correlation between aggressive behavior and physiological indicators. Immobile time and the cortisol level were positively correlated. The longer the immobile time, the higher the cortisol level. A longer immobile time implies that fish have a positive stress coping mechanism (Vindas et al. 2017b). The 5-HIAA/5-HT ratio was also positively correlated with the distance to the central point. This is contrary to studies related to the effects of aggressive fish behavior on brain monoaminergic activity (Xu et al. 2020). This result may be explained by the fact that the aggressive behavior indicators in this experiment were more comprehensive and were not limited to the duration and times involved in the mirror test. The distance to the central point is inversely proportional to the aggressive strength, that is, the closer the distance to the central point, the higher the aggressive strength, and the lower the 5-HIAA/5-HT ratio (Colléter and Brown 2011; Dahlbom et al. 2011). Actually, the knowledge on fish behavior and physiologic parameters is still limited. To improve fish welfare and aquaculture practices, future research needs to combine behavioral and physiological parameters to reduce or avoid significant stress during culture.

Declarations

Author Contributions Haixia Li: Conceptualization, Methodology, Investigation and Writing-Original Draft. Zhen Ma: Conceptualization, Resources, Writing-Original Draft, Writing-Review & Editing, Supervision and Funding Acquisition. Jie Wang: Methodology and Investigation. Xu Zhang: Methodology and Investigation. Yu Hu: Methodology and Investigation. Ying Liu: Conceptualization, Resources and Funding Acquisition.

Funding This work was supported by the National Natural Science Foundation of China (42030408; 32102753), the Scientific and Technical Innovation Project of Dalian, Liaoning Province, China (2021JJ13SN72), and by contributions from the AET. Special thanks are also given to the anonymous reviewers for their constructive comments regarding the manuscript.

Data availability statement The data required to reproduce these findings are available upon request by contact with the corresponding author.

Ethical approval Animal care and use protocols were approved by the Dalian Ocean University Institutional Animal Care and Use Committee and followed institutional guidelines.

Consent to participate Not applicable.

Consent for publication All authors review and approve the manuscript for publication.

Conflict of interest The authors declare no competing interests.

Code availability Not applicable.

References

1. Aguiar A, Giaquinto PC (2018) Low cholesterol is not always good: low cholesterol levels are associated with decreased serotonin and increased aggression in fish. Biol Open 7(12):bio030981. https://doi.org/10.1242/bio.030981
2. Ariyomo TO, Carter M, Watt PJ (2013) Heritability of boldness and aggressiveness in the zebrafish. Behav Genet 43(2):161–167. https://doi.org/10.1007/s10519-013-9585-y
3. Backström T, Winberg S (2017) Serotonin coordinates responses to social stress-What we can learn from fish. Front Neurosci 11:595. https://doi.org/10.3389/fnins.2017.00595

4. Bessa E, Sadoul B, Mckenzie DJ, Geffroy B (2021) Group size, temperature and body size modulate the effects of social hierarchy on basal cortisol levels in fishes. Horm Behav 136:105077. https://doi.org/10.1016/j.yhbeh.2021.105077
5. Boscolo CNP, Pereira TSB, Batalhão IG, Dourado PLR, Schlenk D, de Almeida EA (2018) Diuron metabolites act as endocrine disruptors and alter aggressive behavior in Nile tilapia (*Oreochromis niloticus*). Chemosphere 191:832–838. https://doi.org/10.1016/j.chemosphere.2017.10.009
6. Brandão ML, Colognesi G, Bolognesi MC, Costa-Ferreira RS, Carvalho TB, Gonçalves-de-Freitas E (2018) Water temperature affects aggressive interactions in a Neotropical cichlid fish. Neotrop Ichthyol 16(1). https://doi.org/10.1590/1982-0224-20170081
7. Castanheira MF, Herrera M, Costas B, Conceição LE, Martins CI (2013) Linking cortisol responsiveness and aggressive behaviour in gilthead seabream *Sparus aurata*: Indication of divergent coping styles. Appl Anim Behav Sci 143(1):75–81. https://doi.org/10.1016/j.applanim.2012.11.008
8. Cervantes MC, Delville Y (2007) Individual differences in offensive aggression in golden hamsters: a model of reactive and impulsive aggression? Neuroscience 150(3):511–521. https://doi.org/10.1016/j.neuroscience.2007.09.034
9. Clotfelter ED, O'Hare EP, McNitt MM, Carpenter RE, Summers CH (2007) Serotonin decreases aggression via 5-HT1A receptors in the fighting fish *Betta splendens*. Pharmacol Biochem Be 87(2):222–231. https://doi.org/10.1016/j.pbb.2007.04.018
10. Colléter M, Brown C (2011) Personality traits predict hierarchy rank in male rainbowfish social groups. Anim Behav 81(6):1231–1237. https://doi.org/10.1016/j.anbehav.2011.03.011
11. Dahlbom SJ, Lagman D, Lundstedt-Enkel K, Sundström LF, Winberg S (2011) Boldness predicts social status in zebrafish (*Danio rerio*). PLoS ONE 6(8):e23565. https://doi.org/10.1371/journal.pone.0023565
12. Dorfman HM, Meyer-Lindenberg A, Buckholtz JW (2013) Neurobiological mechanisms for impulsive-aggression: the role of MAOA. Neurosci Aggress 17:297–313. https://doi.org/10.1007/7854_2013_272
13. Falsarella DNL, Brandão ML, Gonçalves-de-Freitas E (2017) Fish adjust aggressive behavior to audience size with limited information on bystanders’ fighting ability. Behav Process 142:116–118. https://doi.org/10.1016/j.beproc.2017.07.002
14. Fanning JR, Lee R, Coccaro EF (2020) Neurotransmitter function in impulsive aggression and intermittent explosive disorder. Wiley Int Handb Psychopath Disorders Law 249–269. https://doi.org/10.1002/9781119159322.ch10
15. Fontana BD, Meinerz DL, Rosa LVC, Mezzomo NJ, Silveira A, Giuliani GS, Quadros VA, Filho GLB, Blaser RE, Rosemberg DB (2016) Modulatory action of taurine on ethanol-induced aggressive behavior in zebrafish. Pharmacol Biochem Be 141:18–27. https://doi.org/10.1016/j.pbb.2015.11.011

16. Gerlai R, Lahav M, Guo S, Rosenthal A (2000) Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. Pharmacol Biochem Be 67(4):773–782. https://doi.org/10.1016/S0091-3057(00)00422-6
17. Guo H, Zhang X, Johnsson JI (2017) Effects of size distribution on social interactions and growth of juvenile black rockfish (*Sebastes schlegelii*). Appl Anim Behav Sci 194l:135–142. https://doi.org/10.1016/j.applanim.2017.05.004
18. Haraldstad T, Haugen TO, Kroglund F, Olsen EM, Höglund E (2019) Migratory passage structures at hydropower plants as potential physiological and behavioural selective agents. Roy Soc Open Sci 6(11):190989. https://doi.org/10.1098/rsos.190989
19. Hellinger J, Jägers P, Spoida K, Weiss LC, Mark MD, Herlitze S (2020) Analysis of the territorial aggressive behavior of the bioluminescent flashlight fish photoblepharon steinitzi in the Red Sea. Front in Mar Sci 7:78. https://doi.org/10.3389/fmars.2020.00078
20. Hirschenhauser K, Wittek M, Johnston P, Möstl E (2008) Social context rather than behavioral output or winning modulates post-conflict testosterone responses in Japanese quail (*Coturnix japonica*). Physiol Behav 95(3):457–463. https://doi.org/10.1016/j.physbeh.2008.07.013
21. Hu Y, Liu Y, Zhou C, Li H, Fan J, Ma Z (2021) Effects of food quantity on aggression and monoamine levels of juvenile pufferfish (*Takifugu rubripes*). Fish Physiol Biochem 47(6):1983–1993. https://doi.org/10.1007/s10695-021-01026-4
22. Hubená P, Horký P, Slavík O (2020) Test-dependent expression of behavioral syndromes: A study of aggressiveness, activity, and stress of chub. Aggressive Behav 46(5):412–424. https://doi.org/10.1002/ab.21909
23. Huntingford F, Adams C (2005) Behavioural syndromes in farmed fish: implications for production and welfare. Behaviour, pp 1207–1221. https://www.jstor.org/stable/4536297
24. Hvas M, Folkedal O, Oppedal F (2021) Fish welfare in offshore salmon aquaculture. Rev Aquacult 13(2):836–852. https://doi.org/10.1111/raq.12501
25. Ibabe I (2020) A systematic review of youth-to-parent aggression: conceptualization, typologies, and instruments. Front Psychol 11:577757. https://doi.org/10.3389/fpsyg.2020.577757
26. Koolhaas JM, Bartolomucci A, Buwalda B, de Boer SF, Flügge G, Korte SM, Meerlo P, Murison R, Olivier B, Palanza P, Richter-Levin G, Sgoifo A, Steimer T, Stiedl O, van Dijk G, Wöhr M, Fuchs E (2011) Stress revisited: a critical evaluation of the stress concept. Neurosci Biobehav Rev 35(5):1291–1301. https://doi.org/10.1016/j.neubiorev.2011.02.003
27. Liu ZH, Li YW, Hu W, Chen QL, Shen YJ (2020) Mechanisms involved in tributyltin-enhanced aggressive behaviors and fear responses in male zebrafish. Aquat Toxicol 220:105408. https://doi.org/10.1016/j.aquatox.2020.105408
28. Loveland JL, Uy N, Maruska KP, Carpenter RE, Fernald RD (2014) Social status differences regulate the serotonergic system of a cichlid fish, *Astatotilapia burtoni*. J Exp Biol 217(15):2680–2690. https://doi.org/10.1242/jeb.100685

29. Lü H, Zhang X, Xi D, Gao T (2014) Use of calcein and alizarin red S for immersion marking of black rockfish *Sebastes schlegelii* juveniles. Chin J of Oceanol Limn 32(1):88–98. https://doi.org/10.1007/s00343-014-3022-9

30. Ma Z, Li H, Hu Y, Fan J, Liu Y (2021) Growth performance, physiological, and feeding behavior effect of *Dicentrarchus labrax* under different culture scales. Aquaculture 534:736291. https://doi.org/10.1016/j.aquaculture.2020.736291

31. Manuel R, Boerrigter JGJ, Cloosterman M, Gorissen M, Flik G, van den Bos R, van de Vis H (2016) Effects of acute stress on aggression and the cortisol response in the A frican sharptooth catfish Clarias gariepinus: differences between day and night. J Fish Biol 88(6):2175–2187. https://doi.org/10.1111/jfb.12989

32. Marks C, West TN, Bagatto B, Moore FB (2005) Developmental environment alters conditional aggression in zebrafish. Copeia 2005(4):901–908. https://doi.org/10.1643/0045-8511(2005)005[0901:DEACAI]2.0.CO;2

33. Metcalfe NB, Van Leeuwen TE, Killen SS (2016) Does individual variation in metabolic phenotype predict fish behaviour and performance? J Fish Biol 88(1):298–321. https://doi.org/10.1111/jfb.12699

34. Moltesen M, Laursen DC, Thörnqvist PO, Andersson M, Winberg S, Höglund E (2016) Effects of acute and chronic stress on telencephalic neurochemistry and gene expression in rainbow trout (*Oncorhynchus mykiss*). J Exp Biol 219(24):3907–3914. https://doi.org/10.1242/jeb.139857

35. Moretz JA, Martins EP, Robison BD (2007a) The effects of early and adult social environment on zebrafish (*Danio rerio*) behavior. Environ Biol Fish 80(1):91–101. https://doi.org/10.1007/s10641-006-9122-4

36. Moretz JA, Martins EP, Robison BD (2007b) Behavioral syndromes and the evolution of correlated behavior in zebrafish. Behav ecol 18(3):556–562. https://doi.org/10.1093/beheco/arm011

37. Oliveira RF, Carneiro LA, Canário AV (2005) No hormonal response in tied fights. Nature 437(7056):207–208. https://doi.org/10.1038/437207a

38. Oliveira RF, Silva JF, Simoes JM (2011) Fighting zebrafish: characterization of aggressive behavior and winner–loser effects. Zebrafish 8(2):73–81. https://doi.org/10.1089/zeb.2011.0690

39. Oliveira RF, Simões JM, Teles MC, Oliveira CR, Becker JD, Lopes JS (2016) Assessment of fight outcome is needed to activate socially driven transcriptional changes in the zebrafish brain. P Natl A Sci 113(5):E654–E661. https://doi.org/10.1073/pnas.1514292113

40. Øverli Ø, Harris CA, Winberg S (1999) Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. Brain Behav Evolut 54(5):263–275. https://doi.org/10.1159/000006627

41. Paull GC, Filby AL, Giddins HG, Coe TS, Hamilton PB, Tyler CR (2010) Dominance hierarchies in zebrafish (*Danio rerio*) and their relationship with reproductive success. Zebrafish 7(1):109–117. https://doi.org/10.1089/zeb.2009.0618

42. Qian ZM, Chen YQ (2017) Feature point based 3D tracking of multiple fish from multi-view images. PLoS ONE 12(6):e0180254. https://doi.org/10.1371/journal.pone.0180254
43. Quadros VA, Costa FV, Canzian J, Nogueira CW, Rosemberg DB (2018) Modulatory role of conspecific alarm substance on aggression and brain monoamine oxidase activity in two zebrafish populations. Prog Neuro-Psychoph 86:322–330. https://doi.org/10.1016/j.pnpbp.2018.03.018
44. Rault JL, Hintze S, Camerlink I, Yee JR (2020) Positive welfare and the like: Distinct views and a proposed framework. Front Vet Sci 7:370. https://doi.org/10.3389/fvets.2020.00370
45. Sherman GD, Mehta PH (2020) Stress, cortisol, and social hierarchy. Curr Opin Psychol 33:227–232. https://doi.org/10.1016/j.copsyc.2019.09.013
46. Sloman KA, Metcalfe NB, Taylor AC, Gilmour KM (2001) Plasma cortisol concentrations before and after social stress in rainbow trout and brown trout. Physiol Biochem Zool 74(3):383–389. https://doi.org/10.1086/320426
47. Teles MC, Oliveira RF (2016) Quantifying aggressive behavior in zebrafish. Zebrafish. Humana Press, New York, NY, pp 293–305. https://doi.org/ 10.1007/978-1-4939-3771-4_20
48. Vindas MA, Gorissen M, Höglund E, Flik G, Tronci V, Damsgård B, Thörnqvist PO, Nilsen TO, Winberg S, Øverli Ø, Ebbesson LO (2017a) How do individuals cope with stress? Behavioural, physiological and neuronal differences between proactive and reactive coping styles in fish. J Exp Biol 220(8):1524–1532. https://doi.org/10.1242/jeb.153213
49. Vindas MA, Magnhagen C, Brännäs E, Øverli Ø, Winberg S, Nilsson J, Backström T (2017b) Brain cortisol receptor expression differs in Arctic charr displaying opposite coping styles. Physiol Behav 177:161–168. https://doi.org/10.1016/j.physbeh.2017.04.024
50. Way GP, Ruhl N, Snekser JL, Kiesel AL, McRobert SP (2015) A comparison of methodologies to test aggression in zebrafish. Zebrafish 12(2):144–151. https://doi.org/10.1089/zeb.2014.1025
51. Whittaker BA, Consuegra S, de Leaniz CG (2021) Personality profiling may help select better cleaner fish for sea-lice control in salmon farming. Reviews in Aquacuture. https://doi.org/10.1101/2021.05.21.444956
52. Wolkers CPB, Serra M, Barbosa Júnior A, Urbinati EC (2017) Acute fluoxetine treatment increases aggressiveness in juvenile matrinxã (*Brycon amazonicus*). Fish Physiol Biochem 43(3):755–759. https://doi.org/10.1007/s10695-016-0329-9
53. Wrangham RW (2018) Two types of aggression in human evolution. P Natl A Sci 115(2):245–253. https://doi.org/10.1073/pnas.1713611115
54. Xi D, Zhang X, Lü H, Zhang Z (2017a) Cannibalism in juvenile black rockfish, *Sebastes schlegelii* (Hilgendorf, 1880), reared under controlled conditions. Aquaculture 479:682–689. https://doi.org/10.1016/j.aquaculture.2017.07.007
55. Xi D, Zhang X, Lü H, Zhang Z (2017b) Prediction of cannibalism in juvenile black rockfish, *Sebastes schlegelii* (Hilgendorf, 1880), based on morphometric characteristics and paired trials. Aquac Res 48(6):3198–3206. https://doi.org/10.1111/are.13150

56. Xu X, Zhang Z, Guo H, Qin J, Zhang X (2020) Changes in aggressive behavior, cortisol and brain monoamines during the formation of social hierarchy in black rockfish (*Sebastes schlegelii*). Animals 10(12):2357. https://doi.org/10.3390/ani10122357
57. Yuan M, Chen Y, Huang Y, Lu W (2018) Behavioral and metabolic phenotype indicate personality in zebrafish (*Danio rerio*). Front Physiol 9:653. https://doi.org/10.3389/fphys.2018.00653
58. Zabegalov KN, Kolesnikova TO, Khatsko SL, Volgin AD, Yakovlev OA, Amstislavskaya TG, Friend AJ, Bao W, Alekseeva PA, Lakstygal AM, Meshalkina DA, Demin KA, de Abreu MS, Rosemberg DB, Kalueff AV (2019) Understanding zebrafish aggressive behavior. Behav Process 158:200–210. https://doi.org/10.1016/j.beproc.2018.11.010
59. Zhang Z, Fu Y, Zhang Z, Zhang X, Chen S (2021) A Comparative Study on Two Territorial Fishes: The Influence of Physical Enrichment on Aggressive Behavior. Animals 11(7):1868. https://doi.org/10.3390/ani11071868
60. Zhang Z, Xu X, Wang Y, Zhang X (2020) Effects of environmental enrichment on growth performance, aggressive behavior and stress-induced changes in cortisol release and neurogenesis of black rockfish *Sebastes schlegelii*. Aquaculture 528:735483. https://doi.org/10.1016/j.aquaculture.2020.735483
61. Zhu W, Zhou X, Xia LX (2019) Brain structures and functional connectivity associated with individual differences in trait proactive aggression. Sci Rep 9(1):1–12. https://doi.org/10.1038/s41598-019-44115-4

Figures

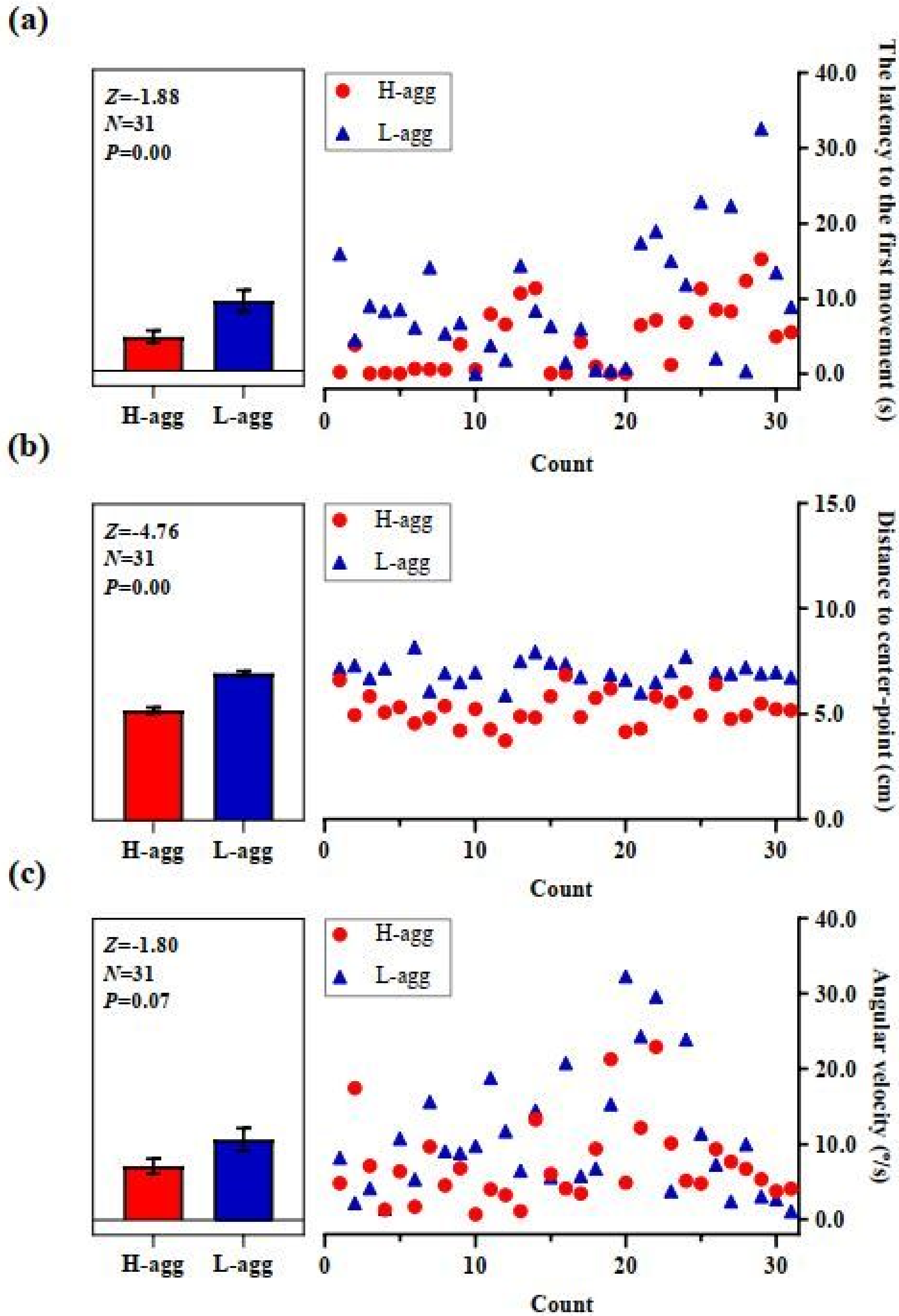

Figure 1

Location selection in H-agg and L-agg fish. (a) Latency to first move; (b) Distance to center-point; (c) Angular velocity. Significant differences are detected by Wilcoxon signed-rank test ($P \leq 0.05$). Values are means ± S.E. (N=31).

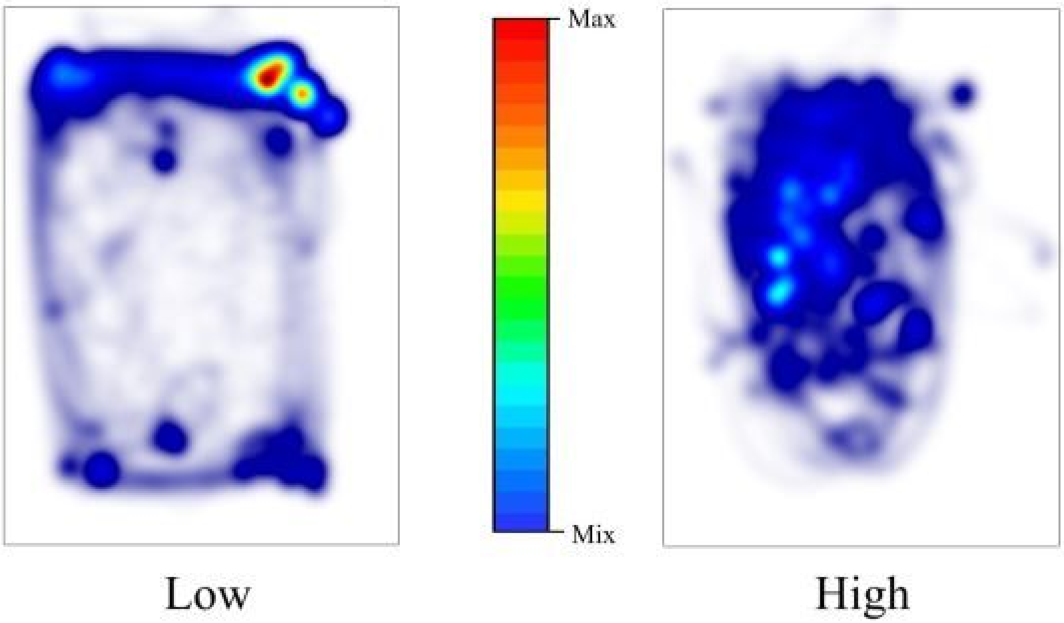

Figure 2

The heat map location of H-agg and L-agg fish.

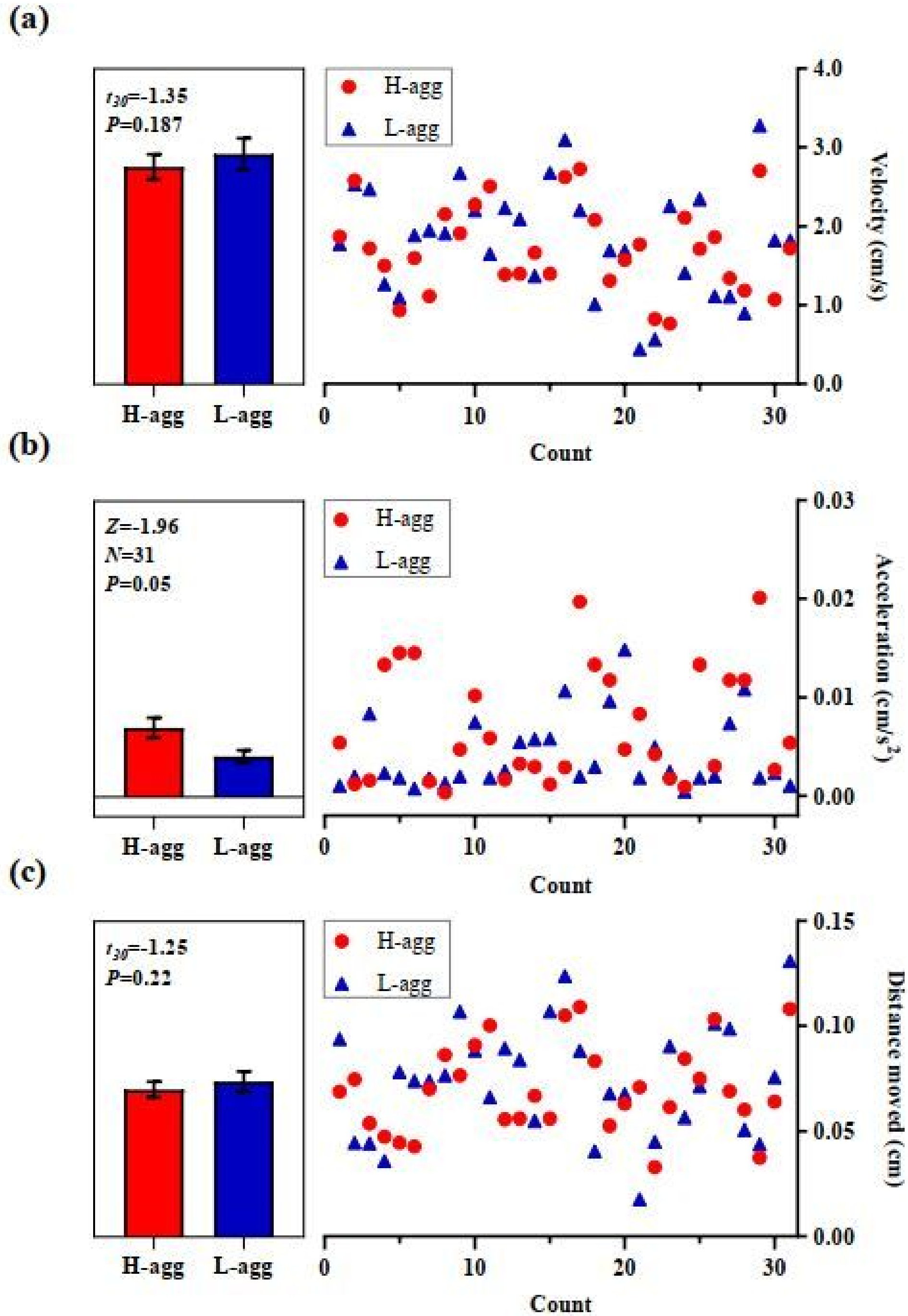

Figure 3

Motion state in H-agg and L-agg fish. (a) Velocity; (b) Acceleration; (c) Distance moved. Significant differences of velocity and distance moved are detected by Paired t test. Significant difference of acceleration is detected by Wilcoxon signed-rank test ($P \leq 0.05$). Values are mean ± S.E. (N=31).

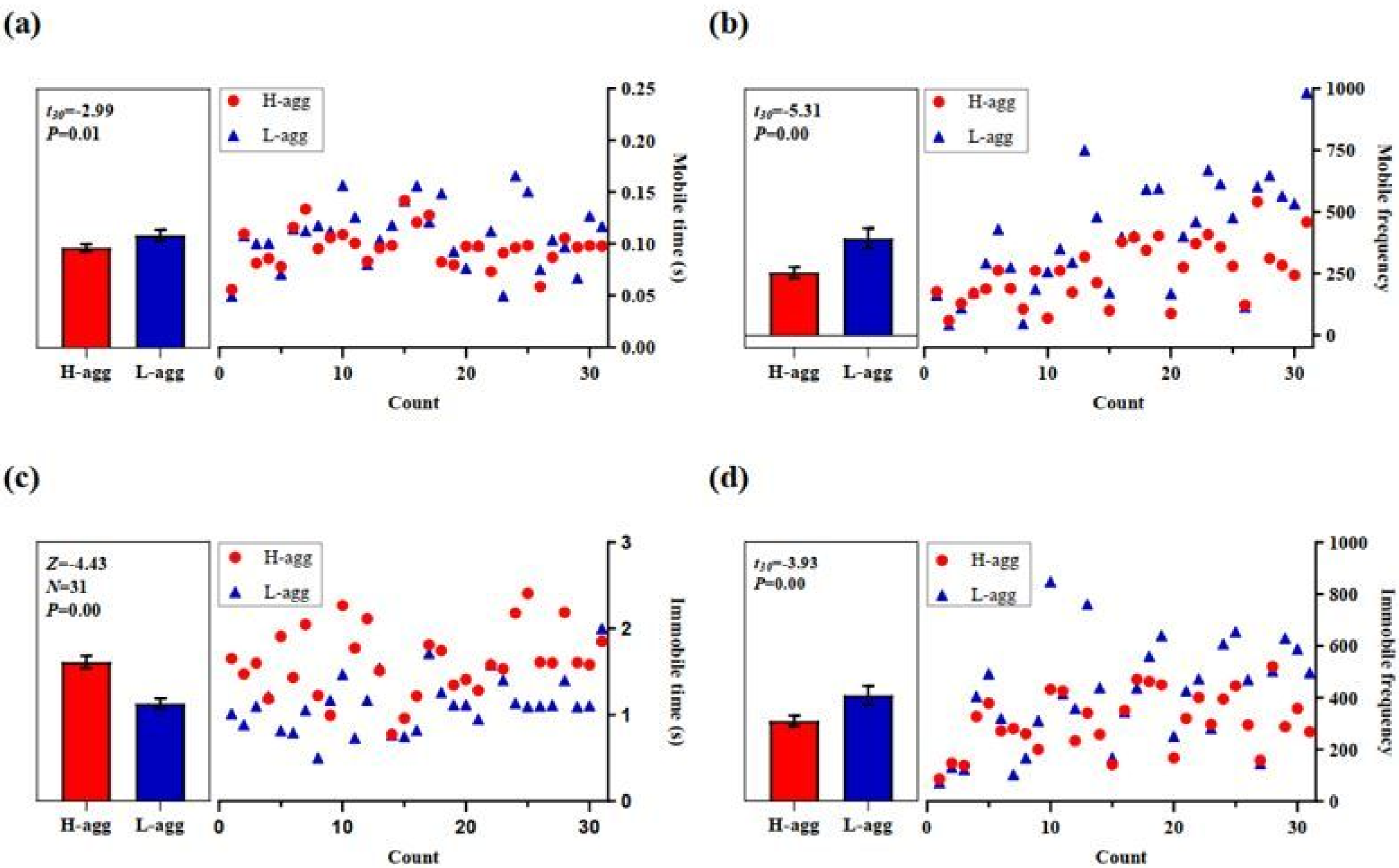

Figure 4

Physical status in H-agg and L-agg fish. (a) Mobile time; (b) Mobile frequency; (c) Immobile time; (d) Immobile frequency. Significant differences of mobile time, mobile frequency and immobile frequency are detected by Paired t test. Significant difference of immobile time is detected by Wilcoxon signed-rank test ($P \leq 0.05$). Values are means ± S.E. (N=31).

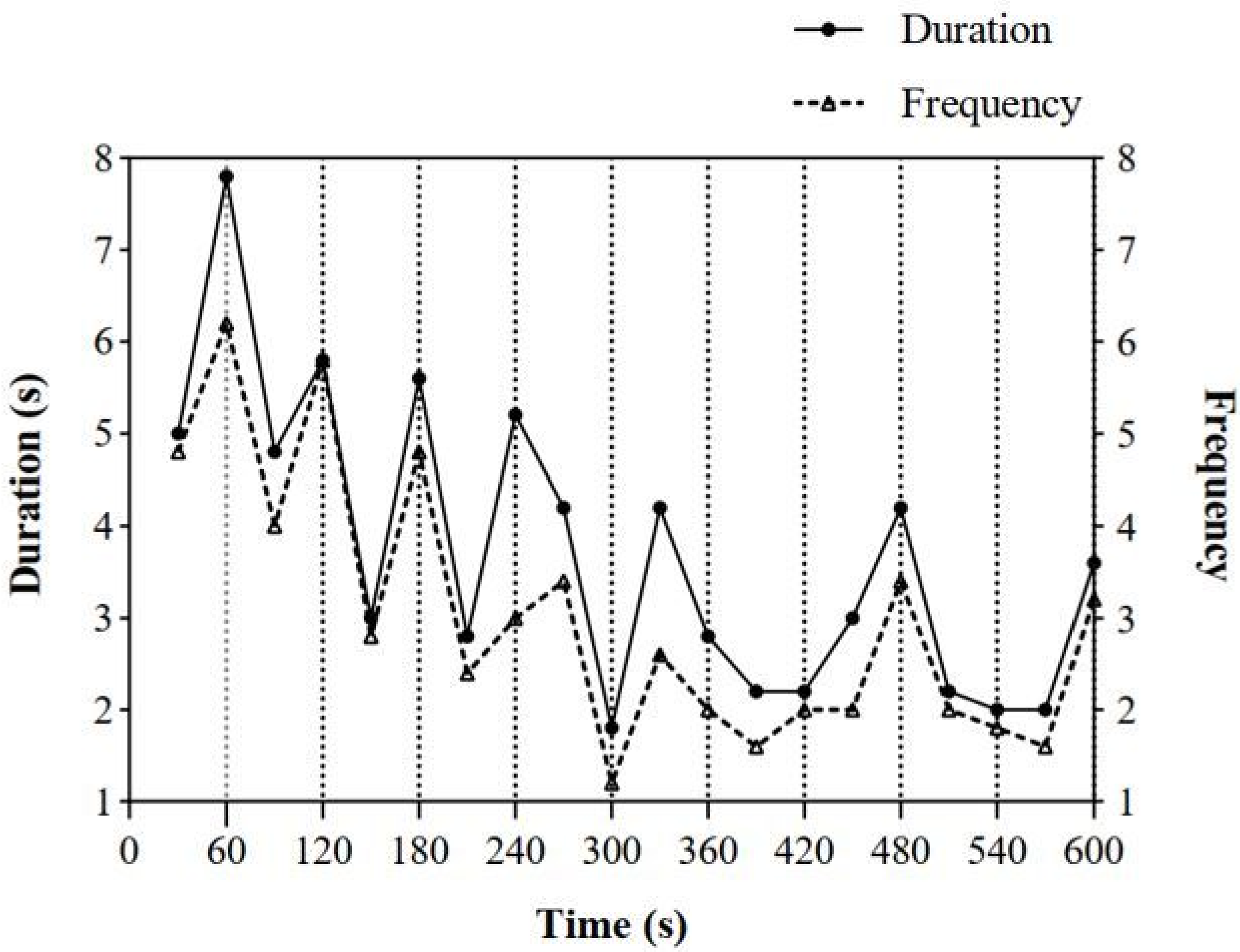

Figure 5

The frequency and duration of individuals with high aggression.

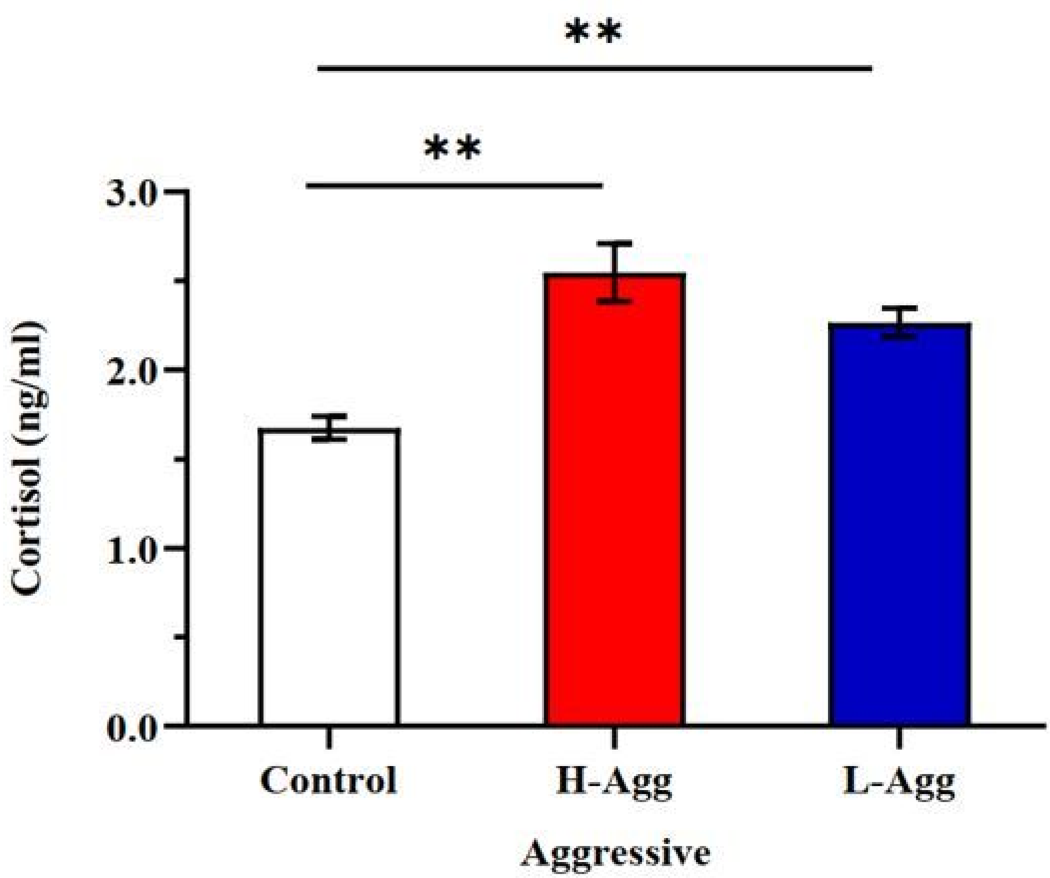

Figure 6

The cortisol levels in H-agg and L-agg fish. Significant differences are detected by one-way ANOVA ($P \leq 0.05$). Values are means ± S.E. (N=27).

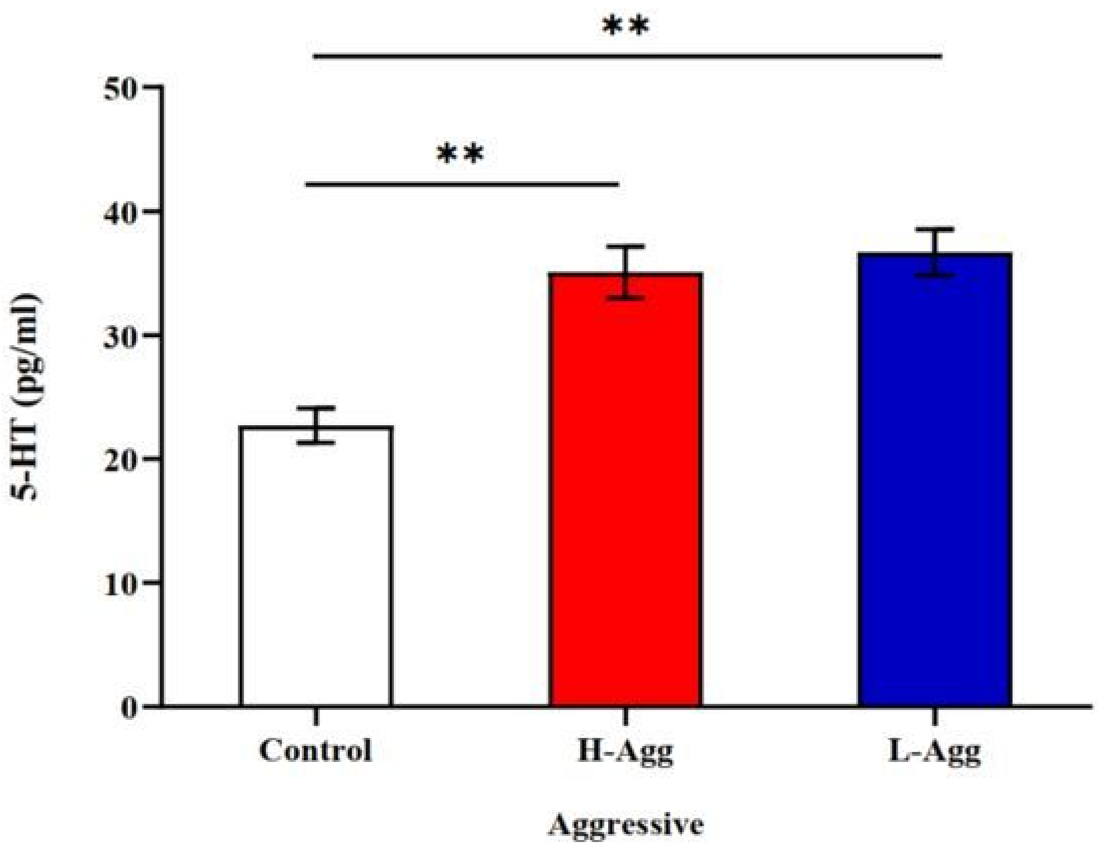

Figure 7

The 5-HT levels in H-agg and L-agg fish. Significant differences are detected by one-way ANOVA ($P \leq 0.05$). Values are means ± S.E. (N=27).

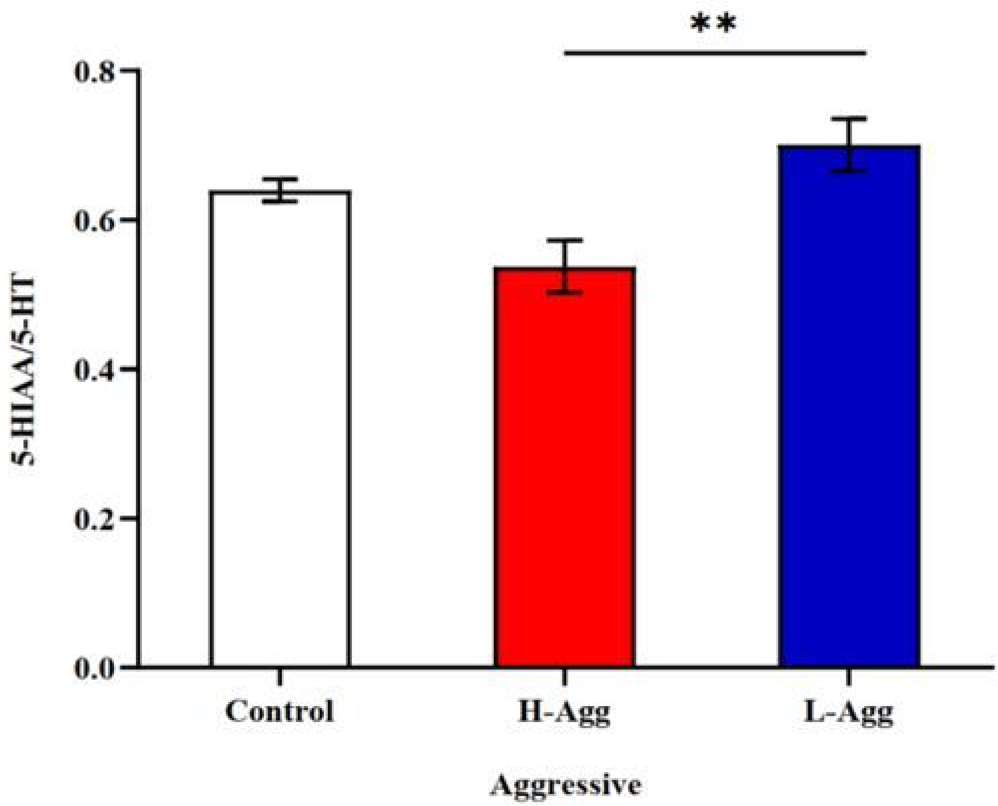

Figure 8

The 5-HIAA/5-HT in H-agg and L-agg fish. Significant differences are detected by one-way ANOVA ($P \leq 0.05$). Values are means ± S.E. (N=27).

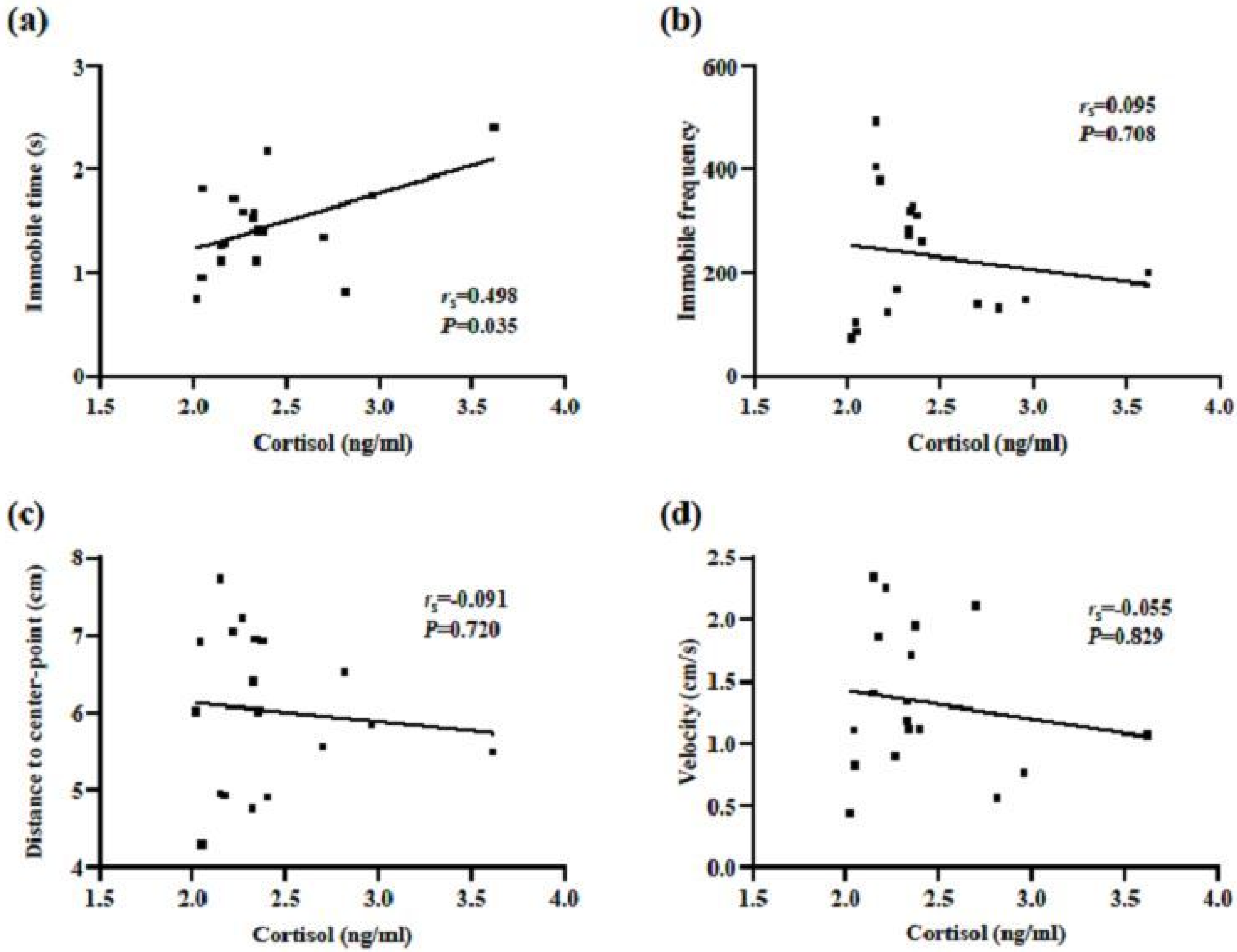

Figure 9

Relationship between cortisol and aggressive behaviors. (a) Immobile time; (b) Immobile frequency; (c) Distance to center-point; (d) Velocity. Spearman r_s and P values are given (N=18).

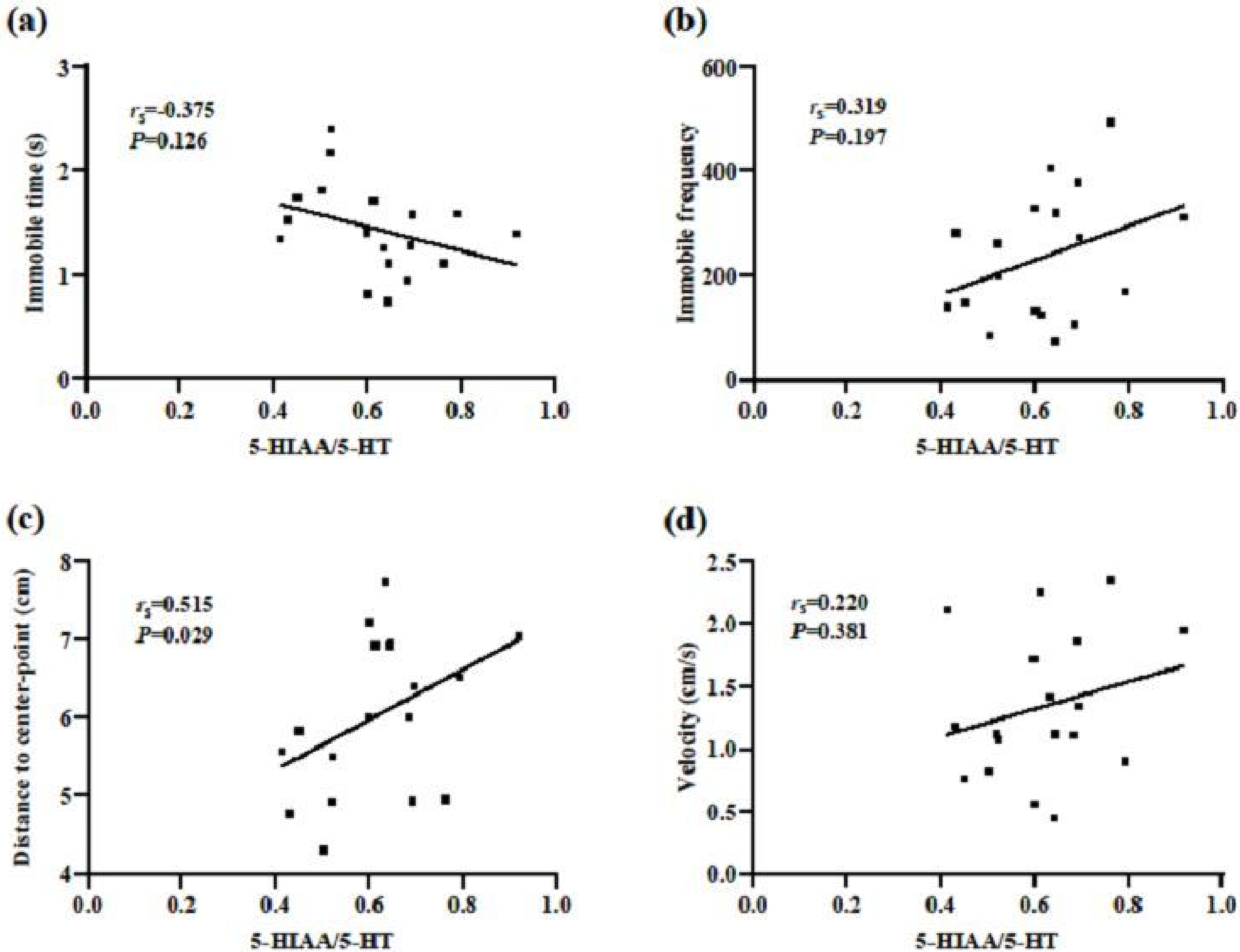

Figure 10

Relationship between 5-HIAA/5-HT and aggressive behaviors. (a) Immobile time; (b) Immobile frequency; (c) Distance to center-point; (d) Velocity. Spearman r_s and P values are given (N=18).