



Sound stimulus effects on dusky damselfish behavior and cognition

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ABSTRACT

Anthropogenic noises are widespread and affect marine wildlife. Despite the growing knowledge on noise pollution in the marine environment, its effects on fish cognition are scarce. Here, we investigated the effects of sound exposure on anxiety-like behavior and memory retention on dusky damselfish *Stegastes fuscus*. The animals were trained in a conditioned place aversion task, and exposed to two daily sessions of music at intensities of 60–70 dBA or 90–100 dBA, while the control group was kept at 42–46 dBA (no music) for five days. After that, fish were tested in the novel tank paradigm and tested for the memory of the aversive task. In the novel tank, animals exposed to sound spent more time still and decreased the distance from the bottom of the tank. Animals also spent more time on the aversive side of the conditioning tank. These results suggest that anthropogenic noise applied through high-intensity music can increase anxiety and decrease memory retention in *S. fuscus*, suggesting the deleterious potential of noise for reef species.

1. Introduction

Noises are unwanted, uncomfortable, and unpleasant sounds in a given site. These sounds are found across different environments and have become increasingly widespread globally. Those caused by human activity, also known as anthropogenic noise, are considered one of the forms of noise pollution by the World Health Organization (Berglund et al., 1995). The effects of noise pollution on wildlife can include physiological and behavioral disturbances, such as increased corticosteroid levels (Tennesen et al., 2014, 2018), confusion in communication patterns (Sun and Narins, 2005; Zhao et al., 2021), as well as reproductive impairments (Injaian et al., 2018). The aquatic environments also suffer from increased incidence and elevated levels of noise. Naturally, the marine environment is full of sounds and fishes perceive it through the otolith organs (sacculle, utricle and lagena) (Fay and Popper, 2000; Popper and Fay, 2011; Webb et al., 2008). However, the increase in population levels in coastal areas (Kummu et al., 2016) has transformed the acoustic landscape of these environments, which has been invaded by sounds of anthropogenic origin (Bittencourt et al., 2020;

Pieretti et al., 2020). In water, the emitted sounds propagate faster than in air and, depending on the frequency, can be perceived at long distances from the source. Maritime traffic, seismic studies, and the construction of oil platforms are some of the primary and most studied sources of underwater noise (Slabbekoorn et al., 2010). Several studies show that anthropogenic noise affects different marine taxa, such as mollusks (André et al., 2011), crustaceans (Filiciotto et al., 2016; Tidau and Briffa, 2019), mammals (Blair et al., 2016) and fish (Fakan and McCormick, 2019; Ferrari et al., 2018; Herbert-Read et al., 2017; Holmes et al., 2017).

Despite the extensive literature on noise pollution in the marine environment, studies investigating the effects of these sounds on the cognitive performance of teleosts are scarce. Among the cognitive processes, skills such as learning and memory are essential for animals, as they help make new decisions, allow individuals to anticipate responses to recurring events, and avoid risk situations (Dall et al., 2005). Diverting attention because of anthropogenic noise can compromise the animal in biologically relevant processes, as demonstrated by Ferrari et al. (2018). These authors showed that the damselfish *Pomacentrus*

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amboinensis exposed to the noise of a boat engine were unable to trigger antipredator responses, putting themselves at risk.

The damselfish family (Pomacentridae) is one of the most conspicuous in reef environments (Allen, 1991). They are animals known for producing and emitting sounds in different behavioral contexts (Parmentier et al., 2016). The species *S. fuscus* is endemic to the Brazilian coast (Allen, 1991) and inhabits shallow rocky reefs (Menegatti et al., 2003). These animals show complex cognitive capacity, being able to learn tasks of different types and store the information learned for long periods, forming long-term memories (Silveira et al., 2015, 2019, 2021).

Other study has sought to understand how sound pollution (music and noise) generated on the continent affect *S. fuscus* behavior (Leduc et al., 2021b). In the present study, the objective was to investigate the effects of music of different intensities on the mnemonic capacity and anxiety-like behavior of the damselfish *Stegastes fuscus*. Although there are no tests of hearing capacity in *Stegastes fuscus*, the related species *Stegastes variabilis* presents an audiogram that varies between 85 and 140 dB re 1 μ Pa (Kenyon, 1996), and intensities above this threshold can be harmful to the auditory system of these animals.

To evaluate the effects of noise on *S. fuscus* behavior, we first trained the animals to associate the black side of a two-chambered tank (black and white) with an aversive stimulus (electroshock) and then exposed them to noise pollution (high and medium intensity carnival music). After five days of exposure to sounds, fish were tested to assess memory and were submitted to a novel tank test to assess anxiety. We hypothesized that the increase in sound intensity would cause a reduction in memory retention time and an increase in the anxiety-like behavior, as it is a potential stressor stimulus.

2. Methodology

2.1. Study strategy

We first trained damselfish *S. fuscus* to associate its preferred area of the tank (black background side) with an aversive stimulus (shock) so that fish avoided entering the dark. After 2 training sections, fish recognized the black area and avoided it, indicating memory of the task. Then, we played carnival music twice a day for 5 days to simulate sound stimulus in the natural ambient during the festival. We used 3 levels of sound intensity: 42–46 dBA (lab noise: control), 60–70 dBA, 90–100 dBA. After that, fish were tested for the aversive memory of the task they were trained for, as well as the level of fish anxiety in a novel tank protocol to check the impacts of sound stimulus.

2.2. Fish collection and laboratory conditions

Adult damselfish (*Stegastes fuscus*) were collected in tidal pools at Praia de Pirambúzios, in Nísia Floresta-RN, with a cast net (3 m in diameter, 10 mm mesh). The animals were placed in 30-L barrels with aerators and transported to the laboratory. They were acclimated in 50-L glass tanks (40 × 25 × 30 cm) for 7 days before the experiments (acclimation period suggested by Silveira et al., 2019). The walls and bottoms of the tanks were covered with wallpaper imitating marine gravel in order to improve welfare conditions. Every 12 tanks constituted a closed recirculating system, in which water passed through a mechanical and biological filter and an ozone unit for disinfection. The animals remained visually isolated to avoid confrontations and physical damage. The system water was prepared with artificial salt and reverse osmosis water (35.2 g of salt for 1 L of water) and kept at 28 °C, pH of 8.2, and salinity between 36 and 38 ppm. The photoperiod was set at 12 L:12D (Light:Dark), with lights on at 7 am. The animals were fed three times a day with frozen *Artemia salina*. All procedures were approved by the Ethics Committee on the Use of Animals (CEUA 002/2022).

2.3. Exposure to sound

The protocol for sound exposure used in this study was adapted from Barcellos et al. (2018). For five days, the animals were submitted to two daily music sessions with 2 h each session. The first sound exhibition session started at 10 am and the second at 2 pm. The selected songs were based on hits usually played during the carnival period, Brazil's most significant popular party (Table 1). In many places, the carnival takes place in coastal regions, where the natural environment of *S. fuscus* is found, and can change the soundscape during its occurrence, as shown by Leduc et al. (2021b). During the Brazilian carnival of Salvador, underwater sound pressure levels at a reef near the festival had an increase of 30 dB re 1 μ Pa₂ and aerial sound pressure levels had an increase of >30 dBA re 20 μ Pa, reaching an average of 90 dBA re 20 μ Pa (Leduc et al., 2021b).

The music was played through a portable speaker (BT Speaker TG-117; T&G, China) located 1 m away from the water recirculation system in which the animals lived. The speaker was supported on a platform with rubber mats at the base to block vibration and avoid interference with the animals. Sound intensities and frequencies were measured outside the tanks (dBA re 20 μ Pa) using the Sound Level Meter application (available on Google Play: <https://play.google.com/store/apps/details?id=com.bolshakovdenis.soundanalyzer>) on a Samsung Galaxy J5 Prime Smartphone (Samsung Brazil, Brasília, Brazil).

The animals were divided into 3 experimental groups: control ($n = 10$), 60–70 dBA ($n = 11$) and 90–100 dBA ($n = 11$), with frequencies varying between 300 and 1200 Hz. The control group was subjected to the same tests and laboratory conditions, without sound exposure at predetermined periods, but with the noise of the laboratory environment (42–46 dBA).

Table 1

Music collection used in the present study. It was played once every stimulation time and totaled 2 h of stimulus. The playlist can be accessed at <https://open.spotify.com/playlist/6EwY08BZHtC3xj68Zo9PPt?si=d9aafdb86ead46df>.

Music	Artist/band
Tô na rua	Ivete Sangalo
Dança	Ivete Sangalo
Levada louca	Banda Eva
Acelera aê	Ivete Sangalo
Abalou	Ivete Sangalo
A galera	Ivete Sangalo
Extravasa	Babado Novo
Na base do beijo	Ivete Sangalo
Recife de fora	Babado Novo
Rock tribal	Babado Novo
Tudo certo na Bahia	Banda Eva, Ninha
Café com pão	Vixe Mainha
Zorra	Timbalada
Beija-flor	Timbalada
O canto da cidade	Daniela Mercury
Bate lata	Banda Beijo
Água mineral	Timbalada
Bola de sabão	Babado Novo
Beleza rara	Banda Eva
Cara caramba ou camaleão	Chiclete com Banana
Manivela	Asa de Águia
Swing da cor	Daniela Mercury
Carro velho	Banda Eva
Pipoca	Ara Ketu
Maimbê dandá	Daniela Mercury, Carlinhos Brown
Toque de timbalero	Timbalada
Prefixo de verão	Banda Mel
Chicletear	Chiclete com Banana, Armandinho
Quero chiclete	Chiclete com Banana
Vai sacudir, vai abalar	Cheiro de Amor
Na Bahia	Ivete Sangalo
Crença e fé	Banda mel
Levada louca	Banda Eva
Sorte grande	Ivete Sangalo

2.4. Aversive conditioning

The aversive conditioning procedure was previously validated for *S. fuscus* (Silveira et al., 2019). The animals were tested in a tank ($40 \times 25 \times 20$ cm, 15 L) divided in half by an opaque partition with a guillotine-type door (10×8 cm, raisable), which allowed the animal to pass between the two sides of the tank. Half of the tank was covered (bottom and sides) with white adhesive paper and the other half with black adhesive paper. On the black side, two iron gratings (mesh size 0.7 cm) were positioned on the lateral walls and connected to an electric shock delivery device (6 V, 1.8 mA, 12.6 W) (Fig. 1a).

The experimental procedure followed 5 phases: training 1, training 2, test, sound exposure, and retest, as explained below. Each animal was tested individually, and always entered the tank on the white side because *S. fuscus* present an innate preference for the black environment (Silveira et al., 2019), thus it would enter the black side when allowed. At each test, the tank water was changed to eliminate the accumulation and possible interference of chemical cues released by the animal.

In training 1 (first day), the animal was placed on the white side of the tank for 60 s. After this period, the guillotined door to the black side was opened, allowing the passage to the other side of the tank. When the fish crossed the door, it was closed, and 2 electroshocks were applied with a 30 s interval between them. Latency to enter the black side was recorded. If passage did not occur within 10 min, the animal was returned to the home tank and removed from the sample.

In training 2 (second day), each animal was placed again on the white side for 60 s. After this period, the guillotined door was raised, and the individual had up to 180 s to pass to the black side. Individuals who did not pass were gently guided to the black side with an acrylic plate. Latency to enter the black side was recorded, or it was registered 180 s for the fish guided to the black side. The same electroshock protocol was applied on the black side, and then the animal was returned to the home tank.

In the test (third day), each fish was placed again on the white side of the tank for 60 s. The door was opened, the animal had free access to both sides of the tank for 10 min, and no electroshock was applied. The behavior of the animals was recorded with a webcam (Logitech C920; Logitech, Lausanne, Switzerland) positioned above (1 m) of the tank. The time spent on the black side of the tank was evaluated as a mnemonic response. Locomotor parameters and latency for entry of the black side were also evaluated.

On the fourth day, sound exposure began (protocol described above). This stage lasted five days, and the 2 groups (60–70 dBA and 90–100 dBA) were exposed to 10 sessions of music, totaling 20 h of sound exposure. The control group was kept under the laboratory noise (42–46 dBA).

On the day after the last sound exposure, the animals were retested in the same experimental tank to assess memory. Every individual was placed back on the white side of the tank for 60 s, the door was raised, and the animal's behavior was recorded for 10 min while freely exploring the tank. The time spent on the black side, locomotor parameters and latency to enter the black side were evaluated.

The behavioral records were analyzed using the Zebtrack/UFRN software (Pinheiro-da-Silva et al., 2017) developed on MATLAB platform (R2014a; Math Works, Natick, United States). The following parameters were evaluated: time spent in the black area, average speed, total distance covered, freezing and latency to pass to the black side on each day. Locomotion increase is associated with stress and avoidance (Kalueff et al., 2013; Silveira et al., 2019) and may indicate the animal's memory of the situation experienced before. Freezing (body immobility with only ventilatory movements) indicates fear/anxiety response (Kalueff et al., 2013; Maximino et al., 2010; Silveira et al., 2019), also suggesting memory of the aversive situation.

2.5. Novel tank

The day after the last sound exposure, anxiety-like behavior was investigated in a novel tank test (Fig. 1b). For this, each fish was individually placed in a never-before-explored tank ($40 \times 25 \times 20$ cm, 15 L), and its behavior was recorded for 10 min. In order to reduce a possible influence of the test order (retest of the conditioning and novel tank test), the animals were randomly placed in the tests: half of the animals went first to the novel tank and then to the memory assessment, and the other half did the way around. The tank water was changed at each test to avoid the accumulation of any chemical clues released by the fish.

The videos were analyzed using the Zebtrack/UFRN software, and the parameters of interest were average speed, maximum speed, distance covered, and freezing. We also evaluated the time each animal spent in the bottom, middle and top regions of the novel tank, the distance from the bottom of the tank, and the latency to enter the top area. The permanence in the upper region of the water column was considered a low anxiety response.

2.6. Statistical analysis

Data were analyzed for normality and homoscedasticity using the Kolmogorov-Smirnov and Levene tests.

Black side entry latency was analyzed for each day between groups using the Kruskal-Wallis test. The other results were analyzed using Two-Way Repeated Measures ANOVA, considering the evaluation day (test or retest) and the experimental group (Control, Music 60–70 dBA and Music 90–100 dBA) as factors. When the result was considered

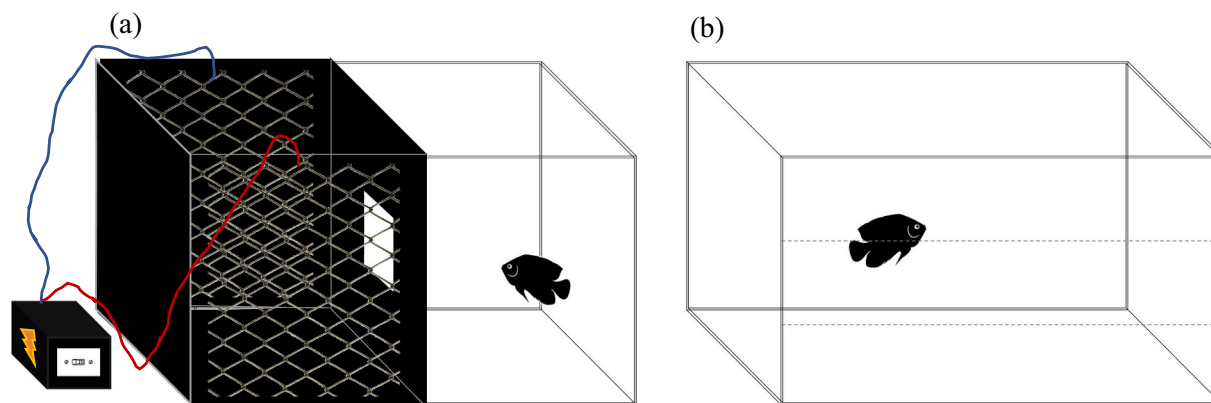


Fig. 1. Schematic view of the tanks used for behavioral tests. (a) Conditioning white and black tank ($40 \times 25 \times 20$ cm), divided in half by a partition with a guillotine door (10×8 cm) and iron grid to electric shock delivery (6 V, 1.8 mA, 12.6 W). (b) Novel tank layout ($40 \times 25 \times 20$ cm) with areas indication (top, middle, and bottom).

significant ($p < 0.05$), the post hoc test applied was Student Newman Keuls (NKS).

Novel tank data (latency to the top and distance from the bottom of the tank) were evaluated between the experimental groups using One Way Anova or Kruskal-Wallis, depending on the parametricity. Two-Way Repeated Measures ANOVA compared the time spent at different tank areas, considering the area (top, middle, and bottom) and group (Control, Music 60–70 dBA, and Music 90–100 dBA). The post hoc Student Newman Keuls (NKS) test was applied in case of statistical significance.

3. Results

3.1. Aversive conditioning

Time spent on the black side of the tank during test and retest (before and after sound exposure) was statistical significance for group (Two-way RM ANOVA, $F_{2,63} = 6.08$, $P = 0.004$), and for experimental day ($F_{1,63} = 17.13$, $P < 0.001$), but no significance was observed for the interaction between group and day ($F_{2,63} = 1.38$, $P = 0.26$). The post hoc SNK test showed that fish from the Music 60–70 dBA and 90–100 dBA groups spent more time on the black side during the retest compared to the control groups ($P < 0.05$) (Fig. 2).

Locomotor parameters during test and retest were compared between groups using Two-way RM ANOVA. For total distance covered, ANOVA did not show significance for group ($F_{2,63} = 0.36$, $P = 0.70$), for day ($F_{1,63} = 0.60$, $P = 0.45$) or for the interaction terms ($F_{2,63} = 0.37$, $P = 0.69$) (Fig. 3a). For moving speed, ANOVA did not show significance for group ($F_{2,63} = 0.36$, $P = 0.70$), for day ($F_{1,63} = 3.37$, $P = 0.07$) or for interaction terms ($F_{2,63} = 2.18$, $P = 0.13$) (Fig. 3b). For the total time in freezing, ANOVA showed significance for day ($F_{1,63} = 5.41$, $P = 0.02$), but there was no significance for group ($F_{2,63} = 0.44$, $P = 0.64$) or the interaction between group and day ($F_{2,63} = 0.03$, $P = 0.97$) (Fig. 3c).

Latency to enter the black side was not statistically significant between groups throughout the experiment (Kruskal-Wallis; Day 1: $H = 0.36$, $df = 2$, $P = 0.84$; Day 2: $H = 3.45$, $df = 2$, $P = 0.18$; Test: $H = 1.73$, $df = 2$, $P = 0.42$; Retest: $H = 0.13$, $df = 2$, $P = 0.94$).

3.2. Novel tank

The time spent at the tank's top, middle, and bottom areas was compared between groups using Two-way RM ANOVA. ANOVA did not show statistical significance for group ($F_{4,95} = 2.65$, $P = 0.09$), but it was significant for area ($F_{4,95} = 9.68$, $P < 0.001$) and for the interaction between group and area ($F_{4,95} = 2.52$, $P = 0.05$). The post hoc SNK test

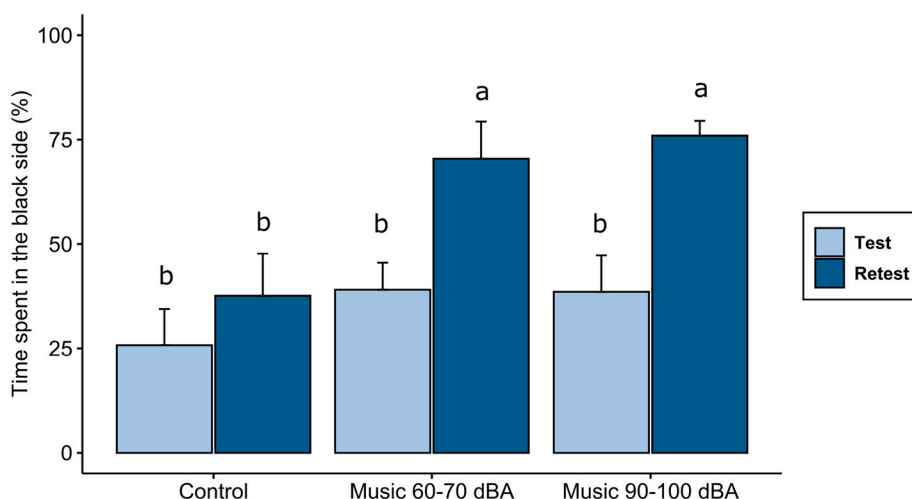


Fig. 2. Time spent by the damselfish on the black side of the aversive conditioning tank. Conditioning was applied by delivering electric shock when fish entered the black side of a white and black box. After 2 training day, receiving shock in the black compartment, fish were tested for memory (Test). Fish were then exposed to sound at 60–70 dBA ($n = 11$), 90–100 dBA ($n = 11$) or 42–46 dBA ($n = 10$, control group) for 5 days, and memory test was redone on the following day (Retest). Bars are mean \pm SEM. Different letters indicate a statistical significance between groups (Two-Way RM ANOVA followed by SNK, $p < 0.05$).

showed that fish from the Music 60–70 dBA and Music 90–100 dBA groups spent less time in the top area of the tank ($P < 0.05$) than the control group (Fig. 4a).

The latency to enter the top area of the tank was statistically significant between groups (Kruskal-Wallis, $H = 8.04$, $df = 2$, $P = 0.01$). The post hoc SNK test showed that the animals from the Music 60–70 dBA group took longer to reach the top area of the tank when compared to the control group ($P < 0.05$) (Fig. 4b).

For distance from the bottom of the tank, One-way ANOVA showed statistical significance ($F_{2,31} = 5.90$, $P = 0.007$). The SNK post hoc showed that fish from the Music 60–70 dBA and Music 90–100 dBA groups were closer to the bottom of the tank compared to the control group ($P < 0.05$) (Fig. 4c).

For freezing, One-way ANOVA presented statistical significance between groups ($F_{2,31} = 6.04$, $P = 0.005$). The post hoc SNK test showed that fish from the Music 60–70 dBA and Music 90–100 dBA groups spent more time still than fish from the control group ($P < 0.05$) (Fig. 4d).

Locomotor parameters were compared between groups using One-way ANOVA. For total distance covered, One-way ANOVA showed statistical significance between groups ($F_{2,31} = 4.58$, $P = 0.02$) and SNK indicated that fish from the Music 60–70 dBA group traveled a shorter distance than fish from the control group ($P < 0.05$) (Fig. 5a). For average speed, One-way ANOVA did not show statistical significance between groups ($F_{2,31} = 0.087$, $P = 0.92$) (Fig. 5b). For maximum speed, Kruskal-Wallis test showed statistical significance ($H = 6.38$ $df = 2$ $P = 0.04$) and SNK test indicated that fish from the Music 90–100 dBA group presented a lower velocity than fish from the control group ($P < 0.05$) (Fig. 5c).

4. Discussion

Here we show that exposure to carnival music between 60 and 100 dB negatively affected the damselfish's behavior. In fact, fish was unable to express the memory of the learned task and showed an increase in anxiety-like parameters. After learning to avoid an area associated to an aversive stimulus (electric shock), fish exposed to 60–70 dBA and 90–100 dBA music returned to the aversive stimulus area. These same animals showed prolonged freezing and shorter distance from the bottom of the tank, indicating a higher level of anxiety. As far as we are aware, this is the first study to show the effects of loud sound stimulus, which propagates between ecosystems (from terrestrial to aquatic environments), on the mnemonic capacity of a reef fish (*S. fuscus*).

The use of music as auditory enrichment in fish has received more attention in recent years. Barcellos et al. (2018) showed that chronic exposure to classical music positively affected zebrafish, reducing

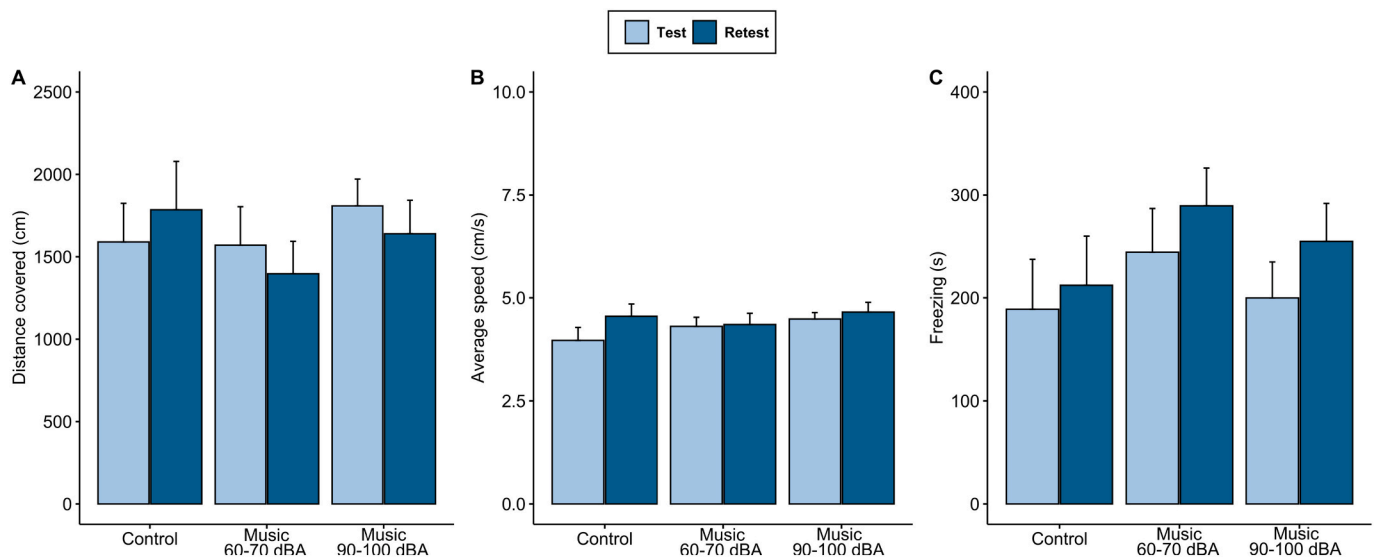


Fig. 3. Damsselfish locomotor parameters on test and retest days of aversive conditioning. Conditioning was applied by delivering electric shock when fish entered the black side of a white and black box. After 2 training day, receiving shock in the black compartment, fish were tested for memory (Test). Fish were then exposed to sound at 60–70 dBA ($n = 11$), 90–100 dBA ($n = 11$) or 42–46 dBA ($n = 10$, control group) for 5 days, and memory test was redone on the following day (Retest). (a) Total distance covered during 10 min, (b) average speed while moving, and (c) time in freezing. Bars are mean \pm SEM. For statistical outcomes, see the Results section.

anxiety-like behavior. Other studies also point to beneficial effects of music on fish development: carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) exposed to classical music have increased daily food intake and gained weight faster (Papoutsoglou et al., 2010, 2013).

Sound enrichment promoted by exposure to moderate intensity music with a high harmony level, such as Mozart, Vivaldi, and Bach, is opposed to auditory stimuli of greater intensity and with frequencies that do not form an acoustic pattern. While classical music can act as a positive stimulus in laboratory or cultivation systems, other types of music can negatively affect fish, both in the laboratory and in natural environments. In areas close to the coast, the noise caused by festivals (carnivals) causes reduction in the foraging efficiency and antipredator response of the damsselfish *S. fuscus* during and after the incidence of sound (Leduc et al., 2021b). In the laboratory, Cartolano et al. (2020) showed that Gulf toadfish (*Opsanus beta*) residing in a laboratory adjacent to the Ultra Music Festival venue had a 4- to 5-fold increase in cortisol levels during the event.

In the present study, damsselfish *S. fuscus* conditioned for place aversion did not avoid the area after five days of sound stimulation. Previous studies by Silveira et al. (2019) showed that damsselfish learn to avoid the location associated with shock and retrieve this memory up to 15 days after the associative training, what was confirmed in this study for the control group. Thus, daily stimulation with music between 60 and 100 dBA may be associated with difficulty in memory formation or information retrieval. Fishes present an elaborated capacity for learning and memory. For instance, fish show appetitive conditioning related to food and conspecific reward (Silveira et al., 2019; Sison and Gerlai, 2010), spatial memory (Darland and Dowling, 2001), avoidance learning (Barcellos et al., 2010) and conspecific recognition (Silveira et al., 2021). However, stress has been indicated as a strong disruptor of learning and memory formation in humans (Schwabe and Wolf, 2010), in rodents (Cohen et al., 2009; el Hage et al., 2006; Park et al., 2008) and also in fishes (Gaikwad et al., 2011; Piato et al., 2011).

Although the discussion of the results shown here may involve acoustic stress on memory formation and retrieval, it is essential to highlight that the damsselfish is a territorial species and usually hides in dens and shelters in the reefs it inhabits. When threatened or under stress, the fish enter its shelter for security. Thus, the longer time spent in the black environment of the experimental tank may be related to the

search for protection and reduced exposure. Benevides et al. (2019) suggest that *S. fuscus* increases time inside refuge during a threat, such as the presence of a diver in its territory. Similar results were observed by Leduc et al. (2021a) for the reef fish *Abudefduf saxatilis*. These authors showed that the fish increased the time in the refuge when exposed to brown noise of 100 dBA in the laboratory. In another study, Leduc et al. (2021b) showed that during the carnival phase in Salvador city, Bahia, the damsselfish *S. fuscus* did not forsake its residence reef even during increased sound incidence. On the other hand, the fish spent less time foraging and decreased antipredator response during high sound impact days.

Despite the adverse effects of music applied here on memory or on the search for refuge (or both), which we still cannot specify, some possible alternatives may include locomotor impediments, reduced motivation, and increased escape response in *S. fuscus*. We observed increased anxiety-like behavior in the novel tank test, indicating the music played here functioned as a stressor element. Regarding locomotor parameters, fish exposed to music showed lower mobility. The animals exposed to the 60–70 dBA presented lower displacement, while those exposed to the 90–100 dBA presented lower maximum velocity than the control fish. The reduction in mobility patterns may indicate fear response in animals (Kalueff et al., 2013). A similar pattern was observed in the white-tailed damsselfish *Pomacentrus chrysurus* (Velasquez Jimenez et al., 2020). When exposed for 5 min to the noise of boat engines (4 strokes) and bulk carriers, these animals reduced the displacement distance. Maximum speed was also reduced for animals exposed to boat engine noise (Velasquez Jimenez et al., 2020).

In summary, sound stress altered locomotor behavior, induced anxiogenic responses, and impaired sensorimotor reaction in the damsselfish *S. fuscus*. The cognitive effects directly provoked by stress or derived from a state of increased anxiety corroborate the modulation of memory evoked by stress, already observed in other species (Gaikwad et al., 2011; Piato et al., 2011). Several studies have focused attention on the cognitive capacity of fish, especially concerning learning and memory retention. Such skills are crucial for the survival of animals, as they imply decision-making in different contexts of the animal's life. Several species of teleost are capable of learning complex and straightforward tasks and forming long-term memories (Salwiczek et al., 2012; Silveira et al., 2015, 2019, 2021; Triki and Bshary, 2020; Truskanov et al.,

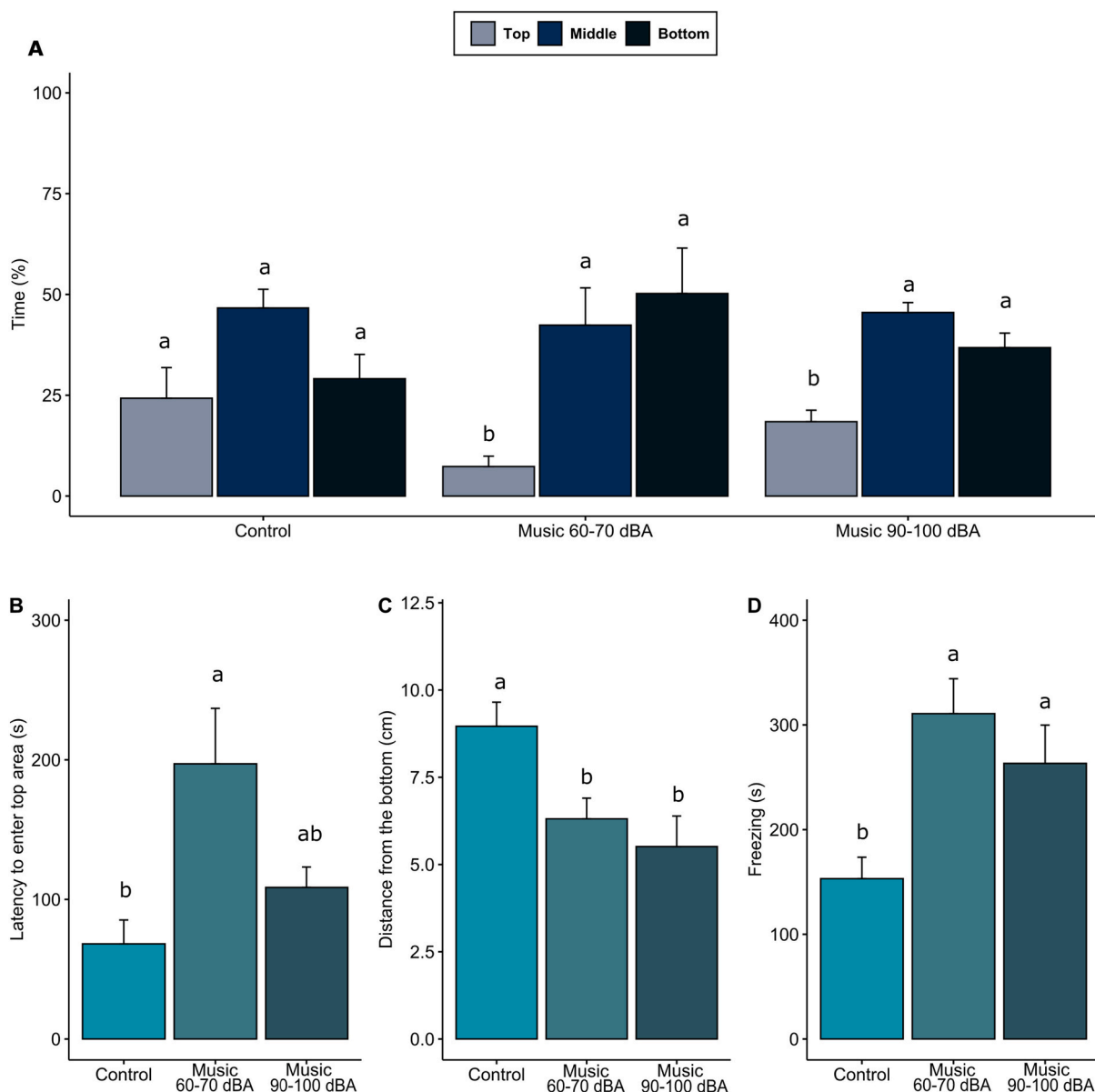


Fig. 4. Damsel fish behavioral parameters during the novel tank test. (a) Tank areas occupation, (b) Latency to the top area, (c) Distance from the bottom, and (d) Freezing. The fish exposed to music played at 60–70 dBA ($n = 11$) or 90–100 dBA ($n = 11$) for 5 days and then tested in the novel tank on the 6th day. The control group received no music treatment and the ambient laboratory sound was recorded at 42–46 dBA ($n = 10$). Increase time at the bottom and higher freezing are indicative of anxiety-like behavior. Bars are mean \pm SEM. (a) different letters indicate a statistical significance between groups (Two-Way RM ANOVA followed by SNK, $p < 0.05$), (b) and (c) different letters indicate a statistical significance between the groups (One-Way ANOVA followed by SNK, $p < 0.05$).

2021). Factors that affect cognitive ability can lead to impairments in the animal's fitness. Deflecting attention between a biologically relevant task and a noise that does not belong to the animal's habitat can lead to an increased risk of predation (Ferrari et al., 2018; Simpson et al., 2016), reduced food intake (Leduc et al., 2021b), or decreased opportunities for courtship and copulation. In this experiment, the sound intensity was measured only in the experimental environment where we kept the fish residence tanks, making it impossible to correctly estimate the intensity level of the acoustic signal transmitted to the animals. However, our results showed the effects of acoustic stress caused by music at both tested intensities on damselfish behavior and cognition. The results presented here highlight the importance of complementary studies to understand better the effects of acoustic signal transmission between ecosystems on the local fauna and the need for noise control at natural sites.

CRedit authorship contribution statement

JFS: Conceptualization, Methodology, Formal analysis, Roles/ Writing – original draft.
 MMS: Methodology, Formal analysis, Writing – review & editing.
 HHAB: Methodology, Supervision.
 LJGB: Conceptualization, Supervision, Writing – review & editing.
 ACL: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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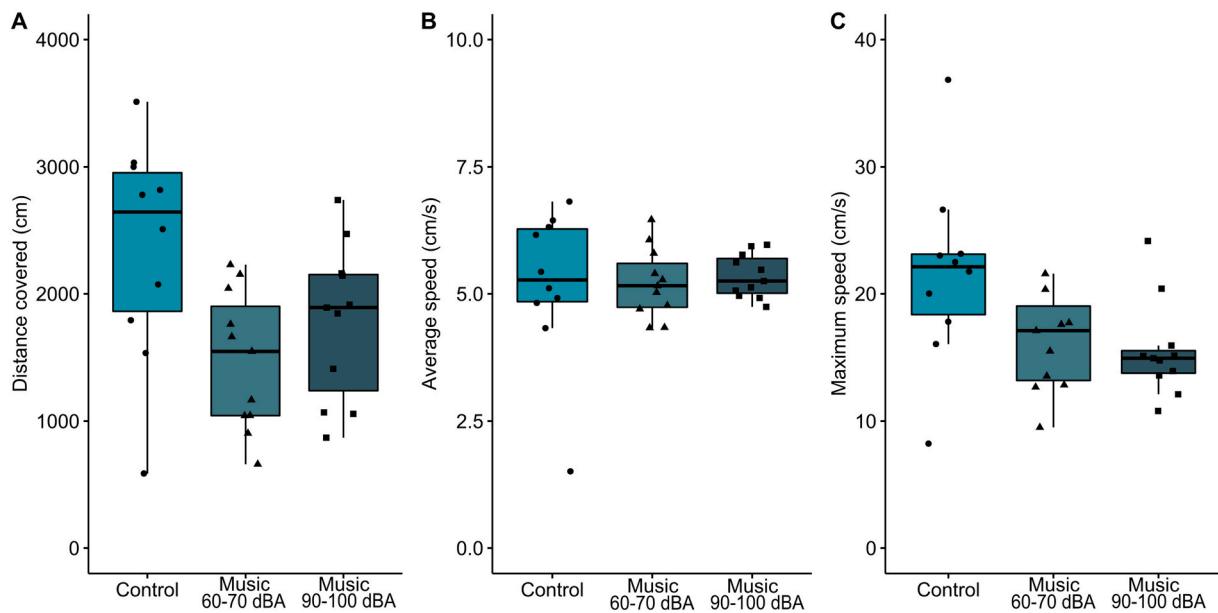


Fig. 5. Damsel fish locomotor parameters in the novel tank test. (a) Total distance covered during 10 min, (b) average speed while moving, and (c) maximum speed achieved in swimming. Fish were exposed to music played at 60–70 dBA (n = 11), 90–100 dBA (n = 11) or ambient noise at 42–46 dBA as control (n = 10) for 5 days. On the 6th day, fish behavior was recorded in the novel tank on the 6th day. Data are presented as median and quartiles. Different letters indicate statistical differences between groups (One-Way ANOVA followed by SNK, $p < 0.05$).

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Declaration of competing interest

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

Data availability

Data will be made available on request.

References

- Allen, G.R., 1991. In: Mergus (Ed.), *Damselfishes of the World*.
 André, M., Solé, M., Lenoir, M., Durfort, M., Quero, C., Mas, A., Lombarte, A., van der Schaar, M., López-Bejar, M., Morell, M., Zaugg, S., Houégnigan, L., 2011. Low-frequency sounds induce acoustic trauma in cephalopods. *Front. Ecol. Environ.* 9 (9), 489–493. <https://doi.org/10.1890/100124>.
 Barcellos, L.J.G., Ritter, F., Kreutz, L.C., Cericato, L., 2010. Can zebrafish *Danio rerio* learn about predation risk? The effect of a previous experience on the cortisol response in subsequent encounters with a predator. *J. Fish Biol.* 76 (4), 1032–1038. <https://doi.org/10.1111/j.1095-8649.2010.02542.x>.
 Barcellos, H.H.A., Koakoski, G., Chaulet, F., Kirsten, K.S., Kreutz, L.C., Kalueff, A.v., Barcellos, L.J.G., 2018. The effects of auditory enrichment on zebrafish behavior and physiology. *PeerJ* 6 (7), e 5162. <https://doi.org/10.7717/peerj.5162>.
 Benevides, L.J., Cardozo-Ferreira, G.C., Ferreira, C.E.L., Pereira, P.H.C., Pinto, T.K., Sampaio, C.L.S., 2019. Fear-induced behavioural modifications in damselfishes can be diver-triggered. *J. Exp. Mar. Biol. Ecol.* 514–515 (December 2018), 34–40. <https://doi.org/10.1016/j.jembe.2019.03.009>.
 Berglund, B., Lindvall, T., Schwela, D., 1995. Guidelines for community noise. World Health Organization. *Noise Vib. Worldw.* 31 (4), 1–141 <https://apps.who.int/iris/handle/10665/66217>. <https://apps.who.int/iris/handle/10665/66217>.
 Bittencourt, L., Barbosa, M., Bisi, T.L., Lailson-Brito, J., Azevedo, A.F., 2020. Anthropogenic noise influences on marine soundscape variability across coastal areas. *Mar. Pollut. Bull.* 160 (May), 111648 <https://doi.org/10.1016/j.marpolbul.2020.111648>.
 Blair, H.B., Merchant, N.D., Friedlaender, A.S., Wiley, D.N., Parks, S.E., 2016. Evidence for ship noise impacts on humpback whale foraging behaviour. *Biol. Lett.* 12 (8), 20160005 <https://doi.org/10.1098/rsbl.2016.0005>.
 Cartolano, M.C., Berenshtein, I., Heuer, R.M., Pasparakis, C., Rider, M., Hammerschlag, N., Paris, C.B., Grosell, M., McDonald, M.D., 2020. Impacts of a local

- music festival on fish stress hormone levels and the adjacent underwater soundscape. *Environ. Pollut.* 265 (Pt A), 114925 <https://doi.org/10.1016/j.envpol.2020.114925>.
 Cohen, H., Liberzon, I., Richter-Levin, G., 2009. Exposure to extreme stress impairs contextual odour discrimination in an animal model of PTSD. *Int. J. Neuropsychopharmacol.* 12 (3), 291–303. <https://doi.org/10.1017/S146114570800919X>.
 Dall, S.R.X., Giraldeau, L.A., Olsson, O., McNamara, J.M., Stephens, D.W., 2005. Information and its use by animals in evolutionary ecology. *Trends Ecol. Evol.* 20 (4), 187–193. <https://doi.org/10.1016/j.tree.2005.01.010>.
 Darland, T., Dowling, J.E., 2001. Behavioral screening for cocaine sensitivity in mutagenized zebrafish. *Proc. Natl. Acad. Sci. U. S. A.* 98 (20), 11691–11696. <https://doi.org/10.1073/pnas.191380698>.
 el Hage, W., Griebel, G., Belzung, C., 2006. Long-term impaired memory following predatory stress in mice. *Physiol. Behav.* 87 (1), 45–50. <https://doi.org/10.1016/j.physbeh.2005.08.039>.
 Fakan, E.P., McCormick, M.I., 2019. Boat noise affects the early life history of two damselfishes. *Mar. Pollut. Bull.* 141 (May 2018), 493–500. <https://doi.org/10.1016/j.marpolbul.2019.02.054>.
 Fay, R.R., Popper, A.N., 2000. Evolution of hearing in vertebrates: the inner ears and processing. *Hear. Res.* 149 (1–2), 1–10. [https://doi.org/10.1016/S0378-5955\(00\)00168-4](https://doi.org/10.1016/S0378-5955(00)00168-4).
 Ferrari, M.C.O., McCormick, M.I., Meekan, M.G., Simpson, S.D., Nedelec, S.L., Chivers, D.P., 2018. School is out on noisy reefs: the effect of boat noise on predator learning and survival of juvenile coral reef fishes. *Proc. R. Soc. B Biol. Sci.* 285 (1871), 20180033. <https://doi.org/10.1098/rspb.2018.0033>.
 Filicetto, F., Vazzana, M., Celi, M., Maccarrone, V., Ceraulo, M., Buffa, G., Arizza, V., de Vincenzi, G., Grammauta, R., Mazzola, S., Buscaino, G., 2016. Underwater noise from boats: measurement of its influence on the behaviour and biochemistry of the common prawn (*Palaemon serratus*, Pennant 1777). *J. Exp. Mar. Biol. Ecol.* 478, 24–33. <https://doi.org/10.1016/j.jembe.2016.01.014>.
 Gaikwad, S., Stewart, A., Hart, P., Wong, K., Piet, V., Cachat, J., Kalueff, A.v., 2011. Acute stress disrupts performance of zebrafish in the cued and spatial memory tests: the utility of fish models to study stress-memory interplay. *Behav. Process.* 87 (2), 224–230. <https://doi.org/10.1016/j.beproc.2011.04.004>.
 Herbert-Read, J.E., Kremer, L., Bruinjtjes, R., Radford, A.N., Ioannou, C.C., 2017. Anthropogenic noise pollution from pile-driving disrupts the structure and dynamics of fish shoals. *Proc. R. Soc. B Biol. Sci.* 284 (1863), 20171627 <https://doi.org/10.1098/rspb.2017.1627>.
 Holmes, L.J., McWilliam, J., Ferrari, M.C.O., McCormick, M.I., 2017. Juvenile damselfish are affected but desensitize to small motor boat noise. *J. Exp. Mar. Biol. Ecol.* 494 (May), 63–68. <https://doi.org/10.1016/j.jembe.2017.05.009>.
 Injaian, A.S., Taff, C.C., Patricelli, G.L., 2018. Experimental anthropogenic noise impacts avian parental behaviour, nestling growth and nestling oxidative stress. *Anim. Behav.* 136, 31–39. <https://doi.org/10.1016/j.anbehav.2017.12.003>.
 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, Z.N.H., 2013. Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. *Zebrafish* 10 (1), 70–86. <https://doi.org/10.1089/zeb.2012.0861>.

- Kenyon, T.N., 1996. Ontogenetic changes in the auditory sensitivity of damselfishes (pomacentridae). *J. Comp. Physiol. A* 179 (4), 553–561. <https://doi.org/10.1007/BF00192321>.
- Kummu, M., de Moel, H., Salvucci, G., Viviroli, D., Ward, P.J., Varis, O., 2016. Over the hills and further away from coast: global geospatial patterns of human and environment over the 20th–21st centuries. *Environ. Res. Lett.* 11 (3), 034010 <https://doi.org/10.1088/1748-9326/11/3/034010>.
- Leduc, A.O.H.C., Costa, J.S.O., do Nascimento Silva, R.R., Winandy, G.S.M., de Araújo, C. B., 2021a. Spatial cognitive abilities of a tide-pool fish show resilience to noise pollution. *J. Ethol.* 39 (2), 225–234. <https://doi.org/10.1007/s10164-021-00697-z>.
- Leduc, A.O.H.C., Nunes, J.A.C.C., de Araújo, C.B., Quadros, A.L.S., Barros, F., Oliveira, H. H.Q., Simões, C.R.M.A., Winandy, G.S.M., Slabbekoorn, H., 2021b. Land-based noise pollution impairs reef fish behavior: A case study with a Brazilian carnival. *Biol. Conserv.* 253 (June 2020), 108910 <https://doi.org/10.1016/j.biocon.2020.108910>.
- Maximino, C., de Brito, T.M., da Silva Batista, A.W., Herculano, A.M., Morato, S., Gouveia, A., 2010. Measuring anxiety in zebrafish: a critical review. *Behav. Brain Res.* 214 (2), 157–171. <https://doi.org/10.1016/j.bbr.2010.05.031>.
- Menegatti, J.v., Vescovi, D.L., Floeter, S.R., 2003. Interações agonísticas e forrageamento do peixe-donzela, *Stegastes fuscus* (Peciformes: Pomacentridae). *Natureza on Line* 1 (2), 45–50.
- Papoutsoglou, S.E., Karakatsouli, N., Papoutsoglou, E.S., Vasilikos, G., 2010. Common carp (*Cyprinus carpio*) response to two pieces of music (“Eine Kleine Nachtmusik” and “Romanza”) combined with light intensity, using recirculating water system. *Fish Physiol. Biochem.* 36 (3), 539–554. <https://doi.org/10.1007/s10695-009-9324-8>.
- Papoutsoglou, S.E., Karakatsouli, N., Skouradakis, C., Papoutsoglou, E.S., Batzina, A., Leondaritis, G., Sakellariadis, N., 2013. Effect of musical stimuli and white noise on rainbow trout (*Oncorhynchus mykiss*) growth and physiology in recirculating water conditions. *Aquac. Eng.* 55, 16–22. <https://doi.org/10.1016/j.aquaeng.2013.01.003>.
- Park, C.R., Zoladz, P.R., Conrad, C.D., Fleshner, M., Diamond, D.M., 2008. Acute predator stress impairs the consolidation and retrieval of hippocampus-dependent memory in male and female rats. *Learn. Mem.* 15 (4), 271–280. <https://doi.org/10.1101/lm.721108>.
- Parmentier, E., Lecchini, D., Mann, D.A., 2016. Biology of damselfishes. In: Fr  d  rich, B., Parmentier, E. (Eds.), *Biology of Damselfishes*. CRC Press. <https://doi.org/10.1201/9781315373874>.
- Piato, A.L., Capiotti, K.M., Tamborski, A.R., Oses, J.P., Barcellos, L.J.G., Bogo, M.R., Lara, D.R., Vianna, M.R., Bonan, C.D., 2011. Unpredictable chronic stress model in zebrafish (*Danio rerio*): behavioral and physiological responses. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 35 (2), 561–567. <https://doi.org/10.1016/j.pnpbp.2010.12.018>.
- Pieretti, N., lo Martire, M., Corinaldesi, C., Musco, L., Dell’Anno, A., Danovaro, R., 2020. Anthropogenic noise and biological sounds in a heavily industrialized coastal area (Gulf of Naples, Mediterranean Sea). *Mar. Environ. Res.* 159 (May), 105002 <https://doi.org/10.1016/j.marenvres.2020.105002>.
- Pinheiro-da-Silva, J., Silva, P.F., Nogueira, M.B., Luchiar, A.C., 2017. Sleep deprivation effects on object discrimination task in zebrafish (*Danio rerio*). *Anim. Cogn.* 20 (2), 159–169. <https://doi.org/10.1007/s10071-016-1034-x>.
- Popper, A.N., Fay, R.R., 2011. Rethinking sound detection by fishes. *Hear. Res.* 273 (1–2), 25–36. <https://doi.org/10.1016/j.heares.2009.12.023>.
- Salwiczek, L.H., Pr  t  t, L., Demarta, L., Proctor, D., Essler, J., Pinto, A.I., Wismer, S., Stoinski, T., Brosnan, S.F., Bshary, R., 2012. Adult cleaner wrasse outperform capuchin monkeys, chimpanzees and Orang-utans in a complex foraging task derived from cleaner – client reef fish cooperation. *PLoS ONE* 7 (11), e49068. <https://doi.org/10.1371/journal.pone.0049068>.
- Schwabe, L., Wolf, O.T., 2010. Learning under stress impairs memory formation. *Neurobiol. Learn. Mem.* 93 (2), 183–188. <https://doi.org/10.1016/j.nlm.2009.09.009>.
- Silveira, M.M., Oliveira, J.J., Luchiar, A.C., 2015. Dusky damselfish *Stegastes fuscus* relational learning: evidences from associative and spatial tasks. *J. Fish Biol.* 86 (3), 1109–1120. <https://doi.org/10.1111/jfb.12618>.
- Silveira, M.M., Ferreira de Souza, J., Pires Moreira, A.L., Silva, P.F., Luchiar, A.C., 2019. Memory retention of appetitive and aversive conditioning in the damselfish *Stegastes fuscus*. *J. Fish Biol.* 95 (3), 772–780. <https://doi.org/10.1111/jfb.14063>.
- Silveira, M.M., de Souza, J.F., Araujo-Silva, H., Luchiar, A.C., 2021. Agonistic behavior is affected by memory in the dusky damselfish *Stegastes fuscus*. *Front. Behav. Neurosci.* 15 (August), 1–9. <https://doi.org/10.3389/fnbeh.2021.663423>.
- Simpson, S.D., Radford, A.N., Nedelec, S.L., Ferrari, M.C.O., Chivers, D.P., McCormick, M.I., Meekan, M.G., 2016. Anthropogenic noise increases fish mortality by predation. *Nat. Commun.* 7 <https://doi.org/10.1038/ncomms10544>.
- Sison, M., Gerlai, R., 2010. Associative learning in zebrafish (*Danio rerio*) in the plus maze. *Behav. Brain Res.* 207 (1), 99–104. <https://doi.org/10.1016/j.bbr.2009.09.043>.
- Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C., Popper, A.N., 2010. A noisy spring: the impact of globally rising underwater sound levels on fish. *Trends Ecol. Evol.* 25 (7), 419–427. <https://doi.org/10.1016/j.tree.2010.04.005>.
- Sun, J.W.C., Narins, P.M., 2005. Anthropogenic sounds differentially affect amphibian call rate. *Biol. Conserv.* 121 (3), 419–427. <https://doi.org/10.1016/j.biocon.2004.05.017>.
- Tennessen, J.B., Parks, S.E., Langkilde, T., 2014. Traffic noise causes physiological stress and impairs breeding migration behaviour in frogs. *Conserv. Physiol.* 2 (1) <https://doi.org/10.1093/conphys/cou032>.
- Tennessen, J.B., Parks, S.E., Swierk, L., Reinert, L.K., Holden, W.M., Rollins-Smith, L.A., Walsh, K.A., Langkilde, T., 2018. Frogs adapt to physiologically costly anthropogenic noise. *Proc. R. Soc. B Biol. Sci.* 285 (1891) <https://doi.org/10.1098/rspb.2018.2194>.
- Tidau, S., Briffa, M., 2019. Anthropogenic noise pollution reverses grouping behaviour in hermit crabs. *Anim. Behav.* 151, 113–120. <https://doi.org/10.1016/j.anbehav.2019.03.010>.
- Triki, Z., Bshary, R., 2020. Long-term memory retention in a wild fish species *Labroides dimidiatus* eleven months after an aversive event. *Ethology* 126 (3), 372–376. <https://doi.org/10.1111/eth.12978>.
- Truskanov, N., Emery, Y., Porta, S., Bshary, R., 2021. Configural learning by cleaner fish in a complex biological market task. *Anim. Behav.* 181, 51–60. <https://doi.org/10.1016/j.anbehav.2021.08.023>.
- Velasquez Jimenez, L., Fakan, E.P., McCormick, M.I., 2020. Vessel noise affects routine swimming and escape response of a coral reef fish. *PLoS ONE* 15 (7), e0235742. <https://doi.org/10.1371/journal.pone.0235742>.
- Webb, J.F., Fay, R.R., Popper, A.N., 2008. In: Webb, J.F., Fay, R.R., Popper, A.N. (Eds.), *Fish Bioacoustics*, Vol. 32. Springer, New York. <https://doi.org/10.1007/978-0-387-73029-5>.
- Zhao, L., Wang, T., Guo, R., Zhai, X., Zhou, L., Cui, J., Wang, J., 2021. Differential effect of aircraft noise on the spectral-temporal acoustic characteristics of frog species. *Anim. Behav.* 182, 9–18. <https://doi.org/10.1016/j.anbehav.2021.09.014>.