

Chronic boat noise exposure elevates boldness and anxiety-like behaviour, while impairing learning in juvenile cichlids *Maylandia zebra*

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Abstract

Underwater anthropogenic noise is a pervasive environmental stressor, yet its impact on the ontogeny of cognitive processes and behavioural coping styles in fish remains poorly understood. This study investigates how chronic exposure to boat noise during early development affects the behavioural phenotype and associative learning in juvenile cichlids (*Maylandia zebra*). Using a split-brood design to control for genetic variation, siblings were reared under either control (lab-silent) or intermittent boat noise conditions for 12 weeks. Subjects were subsequently tested to assess boldness, anxiety-like responses, social aggression, and aversive learning performance. Results revealed that chronic noise exposure induced a complex shift in behavioural coping styles: noise-reared juveniles exhibited faster engagement with novel objects (indicating increased risk-taking) but simultaneously displayed heightened anxiety-like avoidance in light/dark preference tests. Strikingly, noise-reared fish showed significantly impaired performance in a colour discrimination learning task conducted under silent conditions. This demonstrates that the cognitive deficit was not a result of acute attentional distraction, but rather a persistent consequence of developmental stress. Conversely, social aggression was unaffected by rearing history and decreased only during acute noise playback. These findings suggest that early-life acoustic stress reshapes the behavioural phenotype towards a high-anxiety, reactive state and imposes lasting costs on cognitive integrity, potentially compromising fitness in noise-polluted ecosystems.

1. Introduction

With increased human activities in aquatic environments over the past several decades, such as shipping, dredging, sonars, seismic airguns, underwater explosions, and construction, anthropogenic noise has become a concerning stressor in aquatic ecosystems (Duarte et al., 2021). An increasing number of studies demonstrate that boat noise can alter fish behaviour and physiology, including auditory function, potentially impairing sensory processing and compromising survival (Popper and Hawkins, 2019). However, despite growing interest in anthropogenic noise, little is known about how chronic exposure affects cognitive development and behavioural traits in juvenile fish. Studying behavioural traits such as anxiety, boldness, aggression, and learning in juvenile fish is essential for understanding how these early life stages cope with environmental challenges, such as boat noise, and support adaptation. For example, these traits may influence survival by affecting risk-taking, predator avoidance, and also the ability to compete for resources (Sih et al., 2004; Huntingford et al., 2010). Early behavioural phenotypes can also have lasting consequences for social structure, foraging success, and population recruitment (Øverli et al., 2006).

Many of behaviours in fish, such as foraging, mate choice, and recognizing prey and predators, rely on learning and memory (Shettleworth, 2001). These cognitive abilities emerge very early in the development of many fish. Zebrafish, for example, can learn classical and operant conditioning tasks from around four weeks of age (Valente et al., 2012). In parallel, studies on the effects of noise pollution on cognition have focused primarily on terrestrial organisms, to date. For example, increased noise

levels have been shown to negatively affect multiple cognitive processes, such as perception, learning and memory, in humans and rodents, (Lercher et al., 2003; Tao et al., 2015). However, the potential impact of anthropogenic noise on the cognitive abilities of aquatic organisms has been underestimated and warrant attention (Ferrari et al., 2018). This is particularly relevant during the early life stages of fish, a crucial period when they are highly susceptible to environmental fluctuations and predation (Houde, 1987; Heg et al., 2004).

Most studies investigating the effects of anthropogenic noise, including boat noise, on the early life stages of fish have focused on short exposure periods (e.g. 5 days, Lara and Vasconcelos, 2021; two weeks, Faria et al., 2022). However, the behavioural and cognitive consequences of longer-term noise exposure (i.e., more than 2 weeks) in young fish remain weakly understood. Furthermore, much of the existing research has focused on larvae or very early juveniles, whose sensory and neural systems are still developing (Majoris et al., 2021; Nawang et al., 2022). By comparison, later juvenile stages exhibit more advanced neural organisation and integrative processing, suggesting that noise exposure during this period may influence behavioural regulation and learning differently to earlier developmental stages. For example, zebrafish preglomerular complex (PG) projection neurons continue to mature into the late juvenile stage (Bloch et al., 2020), which suggests that sensor-relay circuits are not fully established in early juveniles. These developmental differences imply that responses to sustained noise could change throughout ontogeny.

A recent study by Wang et al. (2025) demonstrated that the effects of boat noise on group behaviour in juvenile cichlids *Maylandia zebra*, such as foraging, social interactions, and activity levels, only became significant after four weeks of exposure, with evidence of habituation after 12 weeks. These findings highlight the importance of assessing long-term exposure effects. Here, we used the same model species - a maternal mouthbrooding cichlid that is well known for its complex social dynamics - and a split-brood design to minimize genetic variation, with juvenile siblings from each brood allocated to either control (lab-silent) or boat noise conditions for a 12-week exposure period (as in Wang et al., 2025). After these 12 weeks, we conducted individual behavioural tests on the fish to investigate the effects of chronic boat noise exposure on behavioural traits and cognitive abilities. Each juvenile was assessed using a series of behavioural tests evaluating boldness (novel object exploration), aggression (mirror-induced agonistic displays), anxiety-like responses (light/dark preference), and associative learning. We predicted that chronic boat noise exposure would increase anxiety-like behaviour and impair associative learning. We also investigated the effect of acute noise exposure during testing (boat noise lasting on the order of minutes) and predicted that it would affect boldness, anxiety-like responses, and social aggression.

2. Material and Methods

2.1 Animals

Adult *Maylandia zebra* were purchased from local fish supplier (Oxyfish, Verlinghem, France) and raised in mixed-sex stock tanks (L 120 cm, W 60 cm, H 50 cm) at ENES laboratory (University of Saint-Etienne, France). Fish were fed daily with commercial cichlid food (JBL NovoRift sticks and Tetra flakes, Tetra, Melle Germany). All tanks were equipped with an external filter (Rena Filstar xP3, Rena France, Annecy, France), aeration system, and PVC tubes (22 cm long, 10 cm diameter) as shelters. Water was maintained at $25 \pm 1^\circ\text{C}$, pH 8.0, and 12:12h light: dark cycle. Males and females mated freely in the stock tanks and were observed daily to identify mouthbrooding females by their extended lower jaw. Once identified, females were isolated in a floating breeder box (L 30 cm, W 20 cm, H 20 cm) within the stock tank close to the aeration. Full rearing protocols are described by Wang et al. (2025). After three weeks of mouth incubation, juveniles were released by gently opening the female's mouth with round-head tweezers.

A total of 58 post-mouthbrooding *M. zebra* juveniles (1.39 ± 0.07 cm Total Length (TL)), from 4 different females (10.5 cm – 12.9 cm TL; Weight (W): $27 \text{ g} \pm 6 \text{ g}$), were exposed to either a control (C) or boat noise (BN) treatment for 12 weeks prior to behavioural tests. A split-brood design was implemented to minimise genetic effects, whereby each brood was randomly subdivided into two groups. Each group was transferred to one of eight independent rearing aquaria (L 30 cm, W 20 cm, H 20 cm) and was randomly allocated to either BN or C conditions (see below). All rearing aquaria were equipped with sand substrate, heater, aeration, and tubes as shelters. Juveniles were fed daily *ad libitum* with commercial cichlid food (JBL NovoRift sticks and Tetra flakes, Tetra, Melle Germany) at random times during the day.

All experimental procedures complied with the relevant guidelines and regulations, including French national guidelines, permits and regulations for animal care and experimental use (permit no. D42-218-0901, ENES laboratory agreement, Direction Départementale de la Protection des Populations, Préfecture du Rhône and Ministry APAFIS #35404-2022020718278608 v8agreement).

2.2 Treatment setup

The BN treatment used for 12 weeks was described in Wang et al. (2025). In brief, 25 passages of motorboat were recorded in the Grangent lake ($45^\circ 45' 07.54''\text{N}$, $4^\circ 25' 56.47''\text{E}$, Loire, France). These were small recreational boats with outboard engines. The boat noises were recorded using a hydrophone (Aquarian Audio Products H2a-XLR, AFAB Enterprises, Anacortes, WA, USA; sensitivity: $-180 \text{ dB re. } 1 \text{ V } \mu\text{Pa}$ – 1, frequency response within $\pm 4 \text{ dB}$ in the range 20 Hz–4.5 kHz), which was connected to a digital recorder (ZOOM H4 at 48 kHz, 16 bit, Chiyoda-ku, Tokyo). The hydrophone was suspended 1 m below the water surface from a stationary boat. All selected recordings were of boats passing within 10 m of the hydrophone.

Field-recorded boat noises were played back in all eight experimental tanks (4 control and 4 boat noise) using custom-made high-fidelity underwater loudspeakers (Fonseca and Maia Alves, 2012) connected to an amplifier and laptop for sound control. The same loudspeakers were placed in the control aquaria without playbacks. The audio playback was calibrated with a H2A-XLR hydrophone placed 3 cm away and connected to the ZOOM H4 recorder. This recording chain was pre-calibrated using a hydrophone

(Bruel and Kjaer 8104, Naerum, Denmark; sensitivity – 205 dB re. 1 V μ Pa – 1; frequency response from 0.1 Hz to 180 kHz) connected to a sound level meter (Bruel and Kjaer 2238 Mediator, Naerum, Denmark). Measurements were obtained using the LZS setting (RMS sound level with slow time weighting and linear frequency weighting; flat weighting: 6.3 Hz - 20 kHz. Sound Pressure Levels (SPL) of sound playbacks were calculated by integrating the average power spectra of the recorded sound files.

All excerpts of boat noise were played back and adjusted to reach ~ 120 dB re. 1 μ Pa SPL in the range of 0 to 1.5 kHz. In the control aquaria SPL were maintained at approximately 100 dB re. 1 μ Pa in the range of 0 to 1.5 kHz, by replacing one-third of the water every 2 days instead of using filters; the same procedure was applied in the BN treatment. This value reflects the typical background noise levels reported for natural freshwater habitats, which generally range between 60 and 100 dB re 1 μ Pa (Amoser and Ladich, 2005; Wysocki et al., 2007).

We report SPL levels up to 1.5 kHz because although the hearing range of the study species is unknown, cichlids typically detect frequencies up to 1.5 kHz (Maruska et al., 2012). Also note that even though fish are primarily sensitive to particle motion (Popper and Hawkins, 2018), our data are presented in sound pressure levels due to a lack of suitable equipment. Nevertheless, the results remain interpretable from a comparative perspective between noise and control treatments (Wang et al. 2025).

During the 12 weeks of the boat noise exposure period, BN juveniles were exposed to a continuous 24 h sound file. Boat noises were played back from 9 a.m. to 6 p.m., with 6 to 17 random boat noises distributed within each hour. Silence was broadcast in the remaining time. The total duration of boat noise exposure in a whole day was 6248 s (7.23% of a day).

Within each treatment group (BN and C), individuals underwent behavioural assessments in two acute conditions, either BN or C. This resulted in four combinations: BN fish tested in the BN acute condition (BN-BN), BN fish tested in the C acute condition (BN-C), C fish tested in the BN acute condition (C-BN) and C fish tested in the C acute condition (C-C). It should be noted that the term acute is used here to describe treatment exposures lasting a few minutes, as opposed to the 12-week long-term exposure.

2.3 Behavioural response assessment

After the 12-week post-mouthbrooding treatment period, 24 C and 34 BN juveniles underwent four individual tests (described below). Figure 1 shows the timeline of the entire experimental procedure.

After the 12-week treatment, juveniles from the same brood were isolated in individual test aquaria (L 14 cm, W 16 cm, H 13 cm) (Fig. 2A) equipped with sand substrate (Aquasand Nature; Zolux déco, Saintes, France) and aeration, and maintained at $25 \pm 1^\circ\text{C}$ inside an air-conditioned room. The test aquaria were divided into two compartments using a white PVC board. One compartment was designated as the test area, and the other contained a JBL loudspeaker, which was placed in a glass tank and sealed with sound-insulating foam (Fig. 2A). Juveniles were acclimated to these experimental aquaria for 2 days before the first test.

Each juvenile underwent four tests: (1) the novel object test, (2) the mirror test, (3) the light/dark preference test, and (4) the learning test (LT). Each juvenile was tested under two conditions (C and BN), except for the LT, as the negative stimulus used in this test (electric shock) was stressful enough on its own, and the addition of noise during testing could have made it unclear whether behavioural changes were due to the noise or to the learning process itself.

A 10-min interval was allowed between the novel object test and the mirror test. The order of the two tests was randomized for each individual, and the juveniles were not handled between tests. Light/dark preference tests were conducted on the following day after the novel object and mirror tests were finished, in a different aquarium (see Fig. 2B, below), and took two days to complete. The learning test was conducted on the day after all light/dark preference tests were completed. This was done at the end of the behavioural test sequence to avoid the possible effects of the negative stimulus on the preceding tests.

After each test, the water in the aquarium was changed before testing the next individual. This procedure was applied to all tests conducted in our study. At the end of the experimental sequence, the juveniles were returned to the rearing aquaria.

Behavioural tests were conducted in both acute boat noise and control environments. This design enabled us to assess not only whether BN juveniles responded consistently in different acoustic environments and how control juveniles reacted to acute noise exposure during testing. The 25 previously described boat passages were played in a continuous loop during the novel object, mirror test, and light/ dark preference tests, with a random silent interval (10-50s) inserted between successive files. The total duration of the sound file was 2274 s, with boat noise comprising 77% of this total.

2.3.1 Novel test

Before testing began, a camera (HD 1080p C920; Logitech International SA, Lausanne, Switzerland) was positioned 10 cm in front of the test aquarium to record the activity of juveniles. A cylinder (4 cm long, 1.3 cm in diameter; unfamiliar to the fish) was placed at the centre of the aquarium. A line attached to the cylinder kept it upright. The recording lasted 5 min, started when the cylinder was dropped into the aquarium and ended when the fish first touched the cylinder with its mouth or any other part of its body. "Latency to contact the object" was measured using Behavioural Observation Research Interactive Software (BORIS; Friard and Gamba, 2016) to quantify boldness. Such novel object tests are widely used in behavioural ecology, as reduced latencies to objects are commonly interpreted as increased boldness (e.g., Brown et al., 2007; Toms et al., 2010). The black cylinder was removed from the aquarium at the end of the recording. See Table 1 and Fig. 2 for further details on the variables and the experimental setup related to the novel object test.

2.3.2 Mirror test

The mirror and novel object tests were conducted consecutively in the same aquarium. The order of the two tests was randomized for each individual and the tests were separated by a 10-min interval during which the juveniles were not handled. The camera was positioned at the same place, and the boat noise

playback was consistent with that used in the novel object test. A mirror (H 15 cm, W 13 cm) was placed randomly on either the left or right side of the test aquarium (Fig. 2A). Each juvenile was recorded for 10 min, and their aggressiveness was quantified by observing their responses to the mirror. We adapted the aggression assessment protocol developed by Balzarini et al. (2014) to account for the smaller size and behavioural repertoire of juvenile cichlids. Specifically, we selected two prominent behaviours - open-mouth biting and lateral display - for quantification (Table 1), as these were the most commonly observed behaviours among the juveniles. The total number of bites and lateral displays was counted using BORIS and was used to calculate an aggressiveness score, which was defined as the sum of bites (or bite attempts) and lateral displays. The juveniles remained in the same aquarium throughout the novel object and mirror tests, until the start of the light-dark preference test. The water in the aquarium was only changed after the two tests.

Table 1

Variables measured in the four different behavioural tests, significance, and group treatments

Novel Test	Behavioural variable	Description	Behavioural and cognitive trait	First Treatment (during 12 weeks)	Second Treatment (after 12 weeks)
	contact latency	latency to contact the object	boldness	C & BN	C & BN
Mirror Test	biting	number of open-mouth bite attempts	aggression	C & BN	C & BN
	lateral displays	lateral orientation to the opponent with fin spread		C & BN	C & BN
Light/dark preference test	duration in black	time spent on the black side (in seconds)	anxiety-like	C & BN	C & BN
Learning Test	number of trials	number of trials required to avoid the colour significantly	associative learning	C & BN	C

2.3.3 Light/dark preference test

The anxiety-related dark/light preference procedure, which has been widely used in mammals (Bourin and Hascoët, 2003) and zebrafish (Maximino et al., 2010b), was used to assess anxiety in juvenile cichlids. Depending on the species and developmental stage, spending more time in either bright or dark areas, is considered an anxiety-like response, as it indicates that the fish is avoiding a specific area while balancing risk and exploration (Maximino et al., 2010a; Lara and Vasconcelos 2021). We adapted our apparatus from that described in Maximino et al. (2010a). It consisted of an experimental tank (L x W x H: 11 x 9 x 5 cm) divided into one half dark zone and the other half light zone. The experimental tank was

filled with 2 cm of water taken from the behavioural test aquarium corresponding to each fish. It was placed in a water-filled holding tank (at the same height as the experimental tank), with two JBL loudspeakers immersed in the water and placed on either side of the experimental tank (Fig. 2B). These speakers were connected to a Zoom H1N digital audio recorder via a splitter, allowing synchronized playback of the same boat noise file. This setup was similar to that used in the previous two experiments.

The holding tank was placed inside a closed opaque box (L x W x H: 20 x 20 x 22 cm) with two LED lights (6 W, white, AQUAVIE RAN G3, Connaux, France) positioned in opposite corners at the top to reduce reflections. The opaque box was used to eliminate external visual stimuli and ensure controlled lighting conditions during the test. The lights produced an average illumination of approximately 400 lux close to the water surface. A camera was positioned at the top of the box. Recording began immediately after the juvenile was placed in the centre of the experimental tank and lasted for 10 min. The subjects were then carefully returned to their experimental tank after the test. The total time spent in the black section was measured using BORIS software.

2.3.4 Learning test

The learning test setup, adapted from Brock et al. (2017), consisted of a transparent tank (L 28cm H 6 cm, W 19 cm) divided into four equal chambers (Fig. 2C). The bottom of the tank was covered with a wire mesh (mesh size: 0.5 cm) and another grid was submerged in water at the top of the tank. Alligator clips connected both grids to a generator to create a closed electric field within the aquarium, ensuring a uniform distribution of the mild electric stimulus throughout the water column. The same camera used in the previous two tests was positioned above the tank and connected to a laptop for filming. Cichlids possess well-developed colour vision; they have seven unique cone opsin genes, which produce visual pigments sensitive to light from ultraviolet to the red end of the spectrum (Carleton, 2009). To avoid bias due to colour preference during testing, we excluded colours that cichlids are known to prefer or that may evoke aversive or stress-related responses. For example, Egger et al. (2011) showed that East African cichlids (haplochromines) have a strong preference for spots of yellow, orange or red. Here, green and grey were selected as visual discriminative stimuli, with green chosen as the conditioned stimulus (CS) as preliminary experiments indicated that it was more effective for learning than grey. Figure 2D presents the flow diagram of the learning test, which had four phases:

(1) Habituation: Juveniles were gently placed in the tank and allowed to habituate for 10 min. During this time, both green and grey were presented underneath the tank, with each colour occupying half of it. The colour on each side was alternated every 5 min to rule out side preference. This was achieved using a cardboard sheet divided into four sections, matching the four chambers of the learning test tank. Each section was split in half, with one side covered with grey paper and the other with green paper. The reverse side had the same layout, but with the colours swapped, allowing the positions of the colours to be alternated by simply flipping the board over.

(2) Basal Preference assessment: The basal preference phase was identical to the habituation phase, lasting also 10 min. Juvenile's preference for each colour was recorded to exclude those that showed a strong preference for one colour over another (only one individual was excluded due to a preference for green).

(3) Conditioning: Following the basal preference assessment, green was presented across the entire bottom of the tank, and the juveniles were conditioned with a brief, mild electric shock (9V, ~ 1 sec) (adapted from Brock et al., 2017). After the shock, grey was presented on the bottom of the tank for 9 sec.

(4) Probe Preference assessment: Juveniles were observed for 2 min to assess their avoidance of the green zone. A predefined 2 cm "hesitation zone" was established next to the colour boundary on the grey side. Avoidance behaviour was classified according to the following three criteria: (i) head turning within the hesitation zone, (ii) freezing within the hesitation zone, and (iii) completely avoiding the green zone. To prevent side bias, the green and grey zones were interchanged between each cycle of the "probe preference" test (conditioning - probe preference). If a juvenile exhibited avoidance behaviours twice in a row, it was considered to have learnt the association and was removed from the tank. If avoidance was not replicated, testing restarted from the conditioning preference phase to repeat the cycle. The juveniles' learning ability was assessed by the total number of cycles needed to avoid the green zone; fewer cycles indicated faster learning. To minimise undue stress on the subjects, trials were terminated after 22 cycles.

2.4 Statistical analyses

Three main comparisons can be made across these tests. (1) C-C vs BN-C / C-C vs BN-BN, representing the impact of long-term noise exposure; (2) C-C vs C-BN, representing the impact of acute concurrent noise (on the order of minutes) during behavioural tests (3) BN-C vs BN-BN, allowing assessment of whether acute noise exposure during testing adds to the effects of prior long-term noise exposure in juveniles.

As the learning tests for both C and BN juveniles were conducted only under control conditions, results represent the impact of prior long-term noise exposure on learning ability.

To test these comparisons, we used a series of statistical models to determine whether boat noise exposure exerts a sustained effect on individual behavioural responses. First, we assessed the potential influence of boat noise using a series of univariate generalised linear mixed models (GLMMs), with each behavioural score (from novel test, mirror test, and light/ dark preference) serving as the response variable. All models included the combination of treatment and test as fixed effects (four categories: C-C, C-BN, BN-C, and BN-BN), as well as the identity of the mother to control for brood effects (four different females) and group size (two categories: 'small with n = 6 and 8 fish, and 'large' with n = 10 and 11 fish). In addition, all models included the individual identity of each fish as a random effect. In total, 116 scores were obtained across all behaviours.

Second, we examined the potential effects of treatment on juvenile learning using a univariate generalized linear mixed model (GLMM), with number of cycles to learn as the response variable. The model included treatment (C or BN), female identity (four different females), and fish size (in cm) as fixed effects. As in the previous model, fish identity was included as a random factor.

Our analyses were conducted within the Bayesian framework using R v3.6.3 (R Core Team, 2020). Univariate generalized linear mixed models (GLMMs) were fitted with a Poisson distribution (logit link) for all behaviours and number of learning trials using the `MCMCglmm` function from the `mcmcglmm` package (Hadfield, 2010). In each model, we adjusted the number of iterations, the burn-in period, and the thinning interval to ensure an effective sample size greater than 2000 and an autocorrelation level of posterior samples below 0.1. For the prior, we used inverse Wishart and augmented priors for both fixed and random effects (univariate models: $V = 1$, $v = 1$, $\alpha\mu = 0$, $\alpha V = 1000$). To assess convergence across the three MCMC chains per model, we used the Gelman-Rubin diagnostic (`gelman.diag` and `gelman.plot` functions from the 'coda' R package; Plummer et al., 2006). All estimates are presented as posterior modes with associated 95% credible intervals, unless otherwise stated.

3. Results

In the novel object test, only BN juveniles tested under BN acute conditions (BN-BN) took significantly less time to contact the object (275 ± 175 s, mean $\pm$ standard deviation) than control juveniles in the control test (125 ± 75 s; posterior mode [95% CI]: -0.82 [-1.56; -0.08]) (C-C vs BN-BN). No significant differences were found among the other conditions (Fig. 3A).

In the mirror test, only control juveniles tested under noise (C-BN) showed a significantly lower aggression score than the remaining conditions. The other three conditions (C-C, BN-C, BN-BN) showed similar scores (Fig. 3B).

The light/dark preference test revealed that control juveniles under control test spent significantly less time in the black area than BN juveniles in both the control (C-C vs. BN-C, posterior mode [95% CI]: 0.25 [0.05; 0.44]) and BN tests (C-C vs. BN-BN, posterior mode [95% CI]: 0.26 [0.05; 0.44]) (Fig. 3C).

The learning test used the "total number of learning cycles" as a measure of learning ability, with a lower number of cycles indicating faster learning. The results showed that BN juveniles needed significantly more trials to learn to avoid the colour green with electric shock compared to control juveniles (posterior mode [95% CI]: -0.6 [-0.99; -0.22]) (C vs BN; Fig. 4).

Table 2

Behavioural responses of juvenile cichlids to control and boat noise across rearing (long-term) and test (acute) conditions. NT: novel test; MT: mirror test; LDPT: light/dark preference test; LT: learning test. NA indicates that the comparison was not performed.

1	Comparisons	Assessment	NT - boldness	MT - aggressiveness	LDPT - anxiety	LT - learning
	C-C vs. C-BN	Impact of concurrent acute noise	No credible effect	C-BN < C-C (also < BN-C and BN-BN)	No credible effect	NA
2	C-C vs. BN-C C-C vs. BN-BN	Impact of prior long-term noise	C-C < BN-BN	No credible effect	CC < BN-C CC < BN-BN	NA
3	BN-C vs. BN-BN	Additive effect of concurrent noise with prior long-term noise exposure	No credible effect	No credible effect	No credible effect	NA
4	C- vs. BN-	Learning speed	NA	NA	NA	C- > BN-

4. Discussion

This study is one of the first to examine the effects of long-term boat traffic noise on the behaviour and cognitive abilities of fish during their early life stages. Chronic exposure produced persistent changes in juvenile cichlids, increasing boldness and anxiety levels while impairing learning, without altering baseline aggression levels. By contrast, acute noise exposure during testing reduced aggression only in control fish, having no additional effect on noise-reared juveniles. These findings highlight a distinction between developmental (long-term) effects of noise on cognitive domains, and acute effects of noise on social interactions during early life stages.

4.1 Long-term effect (C-C vs. BN-C/ BN-BN and C vs. BN comparisons)

Our results showed that 12 weeks of prior exposure to boat noise increase the boldness and increase anxiety levels of cichlid juveniles, while decreasing their learning ability. Comparatively, 12 weeks of boat noise exposure did not affect their aggressiveness.

Regarding boldness, our results showed that BN juveniles took less time to contact the black cylinder after 12 weeks of boat noise exposure, indicating increased boldness. Similar patterns have been observed in other species, where prior stress or high predation risk leads to more active coping behaviours. Examples include three-spined sticklebacks (*Gasterosteus aculeatus*; Brydges et al., 2008),

guppies (*Poecilia reticulata*: Harris et al., 2010), convict cichlids (*Amatitlania nigrofasciata*: Moscicki et al., 2015), and lizards (*Podarcis* spp.: López et al., 2005). Such increased boldness may reflect a proactive coping strategy whereby individuals exposed to chronic or unpredictable stressors adopt risk-taking behaviour, higher activity levels and quicker decision-making (Buenhombre et al., 2024; Castanheira et al., 2017). Chronic stress may alter the activity of the hypothalamic-pituitary-interrenal (HPI) axis and neuromodulators such as serotonin and dopamine, shifting individuals towards a proactive coping profile (Alfonso et al., 2020; de Abreu et al., 2020; Demin et al., 2021). Alternatively, fish subjected to repeated boat noise may have habituated to the acoustic disturbance, reducing their boldness responses (Cox et al., 2018; Radford et al., 2016; Wang et al., 2025). This would explain the shorter latency to contact the object observed in our experiment. Both of these mechanisms - a shift in physiological coping-style and behavioural habituation - could contribute to the increased boldness observed in BN juveniles.

In terms of anxiety, BN juveniles exposed to boat noise for 12 weeks spent more time in the dark area of the tank than control juveniles, suggesting that they were more stressed. The light/dark preference test is a common tool for assessing anxiety, but its interpretation can vary between species and developmental stages. For juvenile cichlids, a preference for darkness may be a coping strategy in response to stress. Similarly, chronic exposure to continuous white noise affected cortisol levels and disrupted locomotor activity in larval zebrafish, leading to anxiety-like behaviour (Lara and Vasconcelos, 2021). In contrast, adult perch display habituation to stress after being exposed to motorboat noise repeatedly for about 11 days (Johansson et al., 2016). This discrepancy may reflect species-specific and life-stage differences in stress responsiveness. Differences between studies may reflect not only variation in life-history traits and social complexity among species, but also differences in acoustic characteristics (e.g. sound type, intensity, and duty cycle) and in developmental stage at which individuals are exposed (Popper et al., 2019). Notably, the perch examined by Johansson et al. (2016) were adults, whereas our study focused on juveniles, a life stage that may be more sensitive to environmental stressors by nature. Cichlids are known for their complex social structures and pronounced parental investment; these factors likely render juveniles particularly sensitive to environmental perturbations. Compared to other species such as perch, which have simpler life histories and fewer social dependencies, juvenile cichlids appear to be less able to cope with long-term anthropogenic noise.

In addition, our study demonstrates that long-term exposure to noise can disrupt the associative learning of juvenile freshwater cichlids using a colour-cue paradigm. Strikingly, juvenile cichlids exposed to long-term boat noise required more time to learn the colour associated with the aversive stimulus, compared to control juveniles. By contrast, the control fish seemed able to focus more on observing their environment and discovering and completing the learning tasks. This is consistent with the "distracted prey hypothesis", which posits that noise exposure (and environmental pollution in general) can lead to involuntary shifts in attention (Chan et al., 2010), thereby diminishing the cognitive resources available for performing relevant tasks. However, an alternative explanation is that the juveniles exhibited a behavioural inhibition as a consequence of persistently elevated stress due to chronic noise exposure

(Sabet et al., 2016). This behavioural inhibition may reduce exploration and interaction with environmental cues, thereby impairing learning performance. Future studies incorporating measures of activity would help to determine whether reduced learning under noise exposure reflects attentional shifts or stress-induced behavioural suppression.

Previous research on juvenile coral reef fish have shown that boat noise can impair antipredator learning and reduce survival (Ferrari et al., 2018; McCormick et al., 2018). By contrast, previous work on tide-pool juvenile fish found that spatial cognitive abilities were resilient to noise exposure. For example, Leduc et al. (2021) found that Sergeant Major (*Abudefduf saxatilis*) juveniles exposed to additional motorboat noise (~ 100 dBA vs 45 dBA control) in a T-maze task showed no impairment in spatial learning or memory performance. These contrasting results may be explained by the habitat in which the fish were caught, as tide pools and rocky reefs are among the noisiest habitats in the aquatic realm (Lugli, 2010). Nevertheless, de Souza et al. (2022) found that anthropogenic noise exposure impaired the cognition of adult dusky damselfish (*Stegastes fuscus*), which were collected from rocky tide pools in a similar manner. Taking into account the variation in cognitive tasks, evolutionary life histories (i.e., whether species evolved in particularly noisy habitats) and life stages investigated in different studies, it is likely that juveniles across species are likely broadly susceptible to the cognitive impacts of anthropogenic noise. Moreover, work on Picasso triggerfish (*Rhinecanthus aculeatus*) shows that experimental context, including captivity and prior experience, can affect behaviour and cognition (Cordery et al., 2026). This suggests that differences among studies may also arise from experimental conditions. Future studies should compare laboratory-reared and wild fish to test whether long-term noise effects observed in the lab reflect responses in natural populations.

Previous reviews of fish cognition have highlighted that learning is only one component of a broader cognitive framework, which also includes numerical, spatial, and social cognition (Salena et al., 2021). The present study focused on a single aspect of cognition, namely associative learning. Future work should therefore aim to assess multiple cognitive domains within the same individuals to determine whether noise effects are general across cognitive functions.

4.2 Effect of boat noise during testing (C-C vs. C-BN comparison)

To evaluate the influence of acute boat noise exposure during testing, C and BN juveniles (aged around 12 weeks post-mouthbrooding) underwent individual testing under C and BN conditions. Behavioural responses were measured using three distinct but complementary tests: the novel object, mirror, and light/dark preference tests.

The results showed that concurrent exposure to boat noise decreases the aggression of cichlid juveniles when they are presented with a mirror. This behavioural suppression likely reflects an acute stress response induced by the sudden onset of noise. Exposure to unpredictable or intense sound can activate the hypothalamic-pituitary-interrenal (HPI) axis, leading to elevated cortisol secretion (Mills et al., 2020; Mommsen et al., 1999). Elevated cortisol levels are known to inhibit aggressive behaviour in several fish

species (Øverli et al., 2002; Gilmour et al., 2005; Castanheira et al., 2013). Under stressful conditions, individuals often reallocate metabolic resources away from energetically costly social or competitive interactions towards behaviours that enhance immediate survival, such as coping and vigilance (Schreck and Tort, 2016). Elevated cortisol may also alter serotonergic and dopaminergic activity in the brain, thereby modulating motivation and reducing the drive for dominance displays (Winberg et al., 1992; Vindas et al., 2018). Therefore, the reduced aggression observed under acute boat noise exposure likely represents a shift in behavioural priorities - from maintaining social status to conserving energy and monitoring environmental threats - in response to a perceived stressor.

Our study found no effect of 10-min short-term noise exposure on boldness or anxiety in 12-week-old juvenile cichlids. This suggests that the acute noise exposure used in our experiment did not elicit a visible stress response. By contrast, Alvey et al. (2007) showed that other cichlids (*A. burtoni*) exhibited a physiological stress response, as determined by elevated whole-body cortisol levels, as early as one week after hatching when larvae were exposed to a 15-min of confinement stress (Alvey et al., 2007).

Exposure to noise has also been found to decrease fish boldness. For example, McCormick et al. (2018) found that Ward's damselfish (*Pomacentrus wardi*) exhibited impaired risk assessment when exposed to repeated passes of motorboats with 30 hp 2-stroke and 4-stroke engines for several minutes per trial. The effects were stronger for 2-stroke engines. This discrepancy with our study may be due to differences in noise exposure duration and intensity, as well as differences in experimental design: in the previous study, noise was applied only before testing, whereas in our study, control juveniles experienced noise only during the behavioural assays. While earlier studies typically used continuous noise exposure lasting up to 24 hours, our experiment involved exposures lasting only a few minutes. Additionally, both the noise level and type in our study differed from those used in previous research: earlier studies used continuous white or motorboat noise at higher intensities (typically ~ 150 dB re. 1 μ Pa), whereas our study employed intermittent boat noise at a lower level (~ 120 dB re. 1 μ Pa) and for a shorter duration. This may have been insufficient to trigger a stress response capable of influencing anxiety or boldness behaviours.

4.3 Additive effect of concurrent noise with prior long-term noise exposure (BN-C vs. BN-BN comparison)

Interestingly, BN juveniles did not appear to be affected by additional short-term exposure to boat noise during testing (BN-C vs. BN-BN), which suggests that boat noise may have a long-lasting effect on them. The absence of further behavioural changes in BN juveniles under acute boat noise exposure indicates behavioural habituation following long-term exposure; they did not show an apparent response during behavioural tests. However, rather than reverting to control-like behaviour, noise-reared fish still appear to display lasting physiological and cognitive changes, as they still differ from control reared fish in terms of boldness and anxiety (Fig. 3A, C). Similar forms of habituation have been observed in fish exposed to prolonged or repeated stress. In such cases, sustained environmental challenges resulted in characteristic and persistent behavioural patterns. For example, chronic stress is recognised as a factor

that can reinforce stable proactive or reactive coping styles, leading to consistent behavioural responses in various contexts (Øverli et al., 2006; Vindas et al., 2017). Long-term stress exposure can also induce enduring changes in serotonergic regulation, producing lasting adjustments in behavioural output (Backström and Winberg, 2017). More generally, chronic stress prompts fish to adopt stable physiological and behavioural states that enable them to function under continued disturbance (Schreck and Tort, 2016). These findings support the interpretation that chronic exposure to boat noise established a stable behavioural state in BN juveniles, allowing them to behave consistently regardless of immediate acoustic conditions, even though their behavioural baseline differed from that of control juveniles.

4.4 Concluding remarks

Collectively, our results highlight the cumulative effects of long-term exposure to boat noise on fish in the early stages of life, which point to increased stress. The increased level of stress could explain the heightened anxiety and boldness behaviour discussed above, which could in turn have led to memory impairment. Since most species depend on learning to perform vital, fitness-related tasks such as finding food, selecting mates and recognizing predators, cognitive abilities (including learning, storing and retrieving information) are absolutely crucial. Noise-induced cognitive deficits and behavioural changes such as those found in the present study could therefore impose significant fitness costs, diminishing survival, growth and subsequent reproduction, especially when exposure occurs during vulnerable early life stages.

Our study demonstrated that exposure to boat noise for 12 weeks, even in the absence of predators and with abundant food, acts as a chronic stressor, significantly altering the boldness, anxiety and learning ability of juvenile cichlids, whereas concurrent acute noise only affected aggression. This early-life vulnerability underscores the importance of investigating the effects of noise during the earliest stages of fish development, and of mitigating noise pollution in aquatic habitats, in order to protect fish populations and ensure their ecological viability. Furthermore, to simulate ecologically relevant conditions, future studies should examine the interactions between noise and other environmental stressors, such as chemical contaminants or predator cues. Importantly, there is also a need for field studies to determine whether the effects observed in the laboratory happen the same in the wild, where environmental complexity and ecological interactions may modulate behavioural and cognitive responses to noise (and other sensory stressors). Ultimately, future research should develop strategies to reduce mono/multisensory pollution in critical habitats, supporting the cognitive health and survival of fish and other wildlife.

Declarations

Ethics approval

This study was conducted at the ENES laboratory, part of the Centre de Recherche en Neurosciences de Lyon at the University of Saint-Étienne, France. Fish housing, breeding conditions, and all experimental

procedures were carried out in accordance with the relevant ethical and regulatory requirements, including APAFIS #35404-2022020718278608 v8 and the ENES agreement: no. G 42-218-0901.

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Conflicts of Interest

The authors declare no conflicts of interest

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Figures

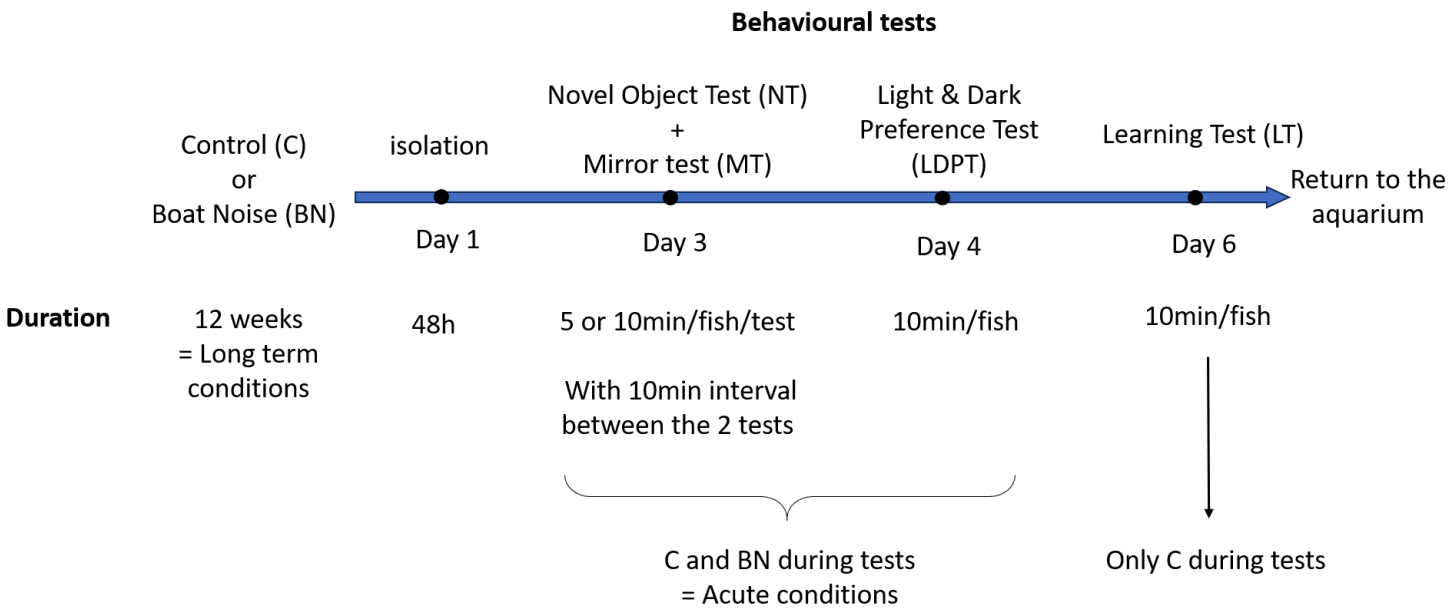


Figure 1

Timeline of the experimental procedure (NT: novel object test; MT: mirror test; LDPT: light/dark preference test; LT: learning test)

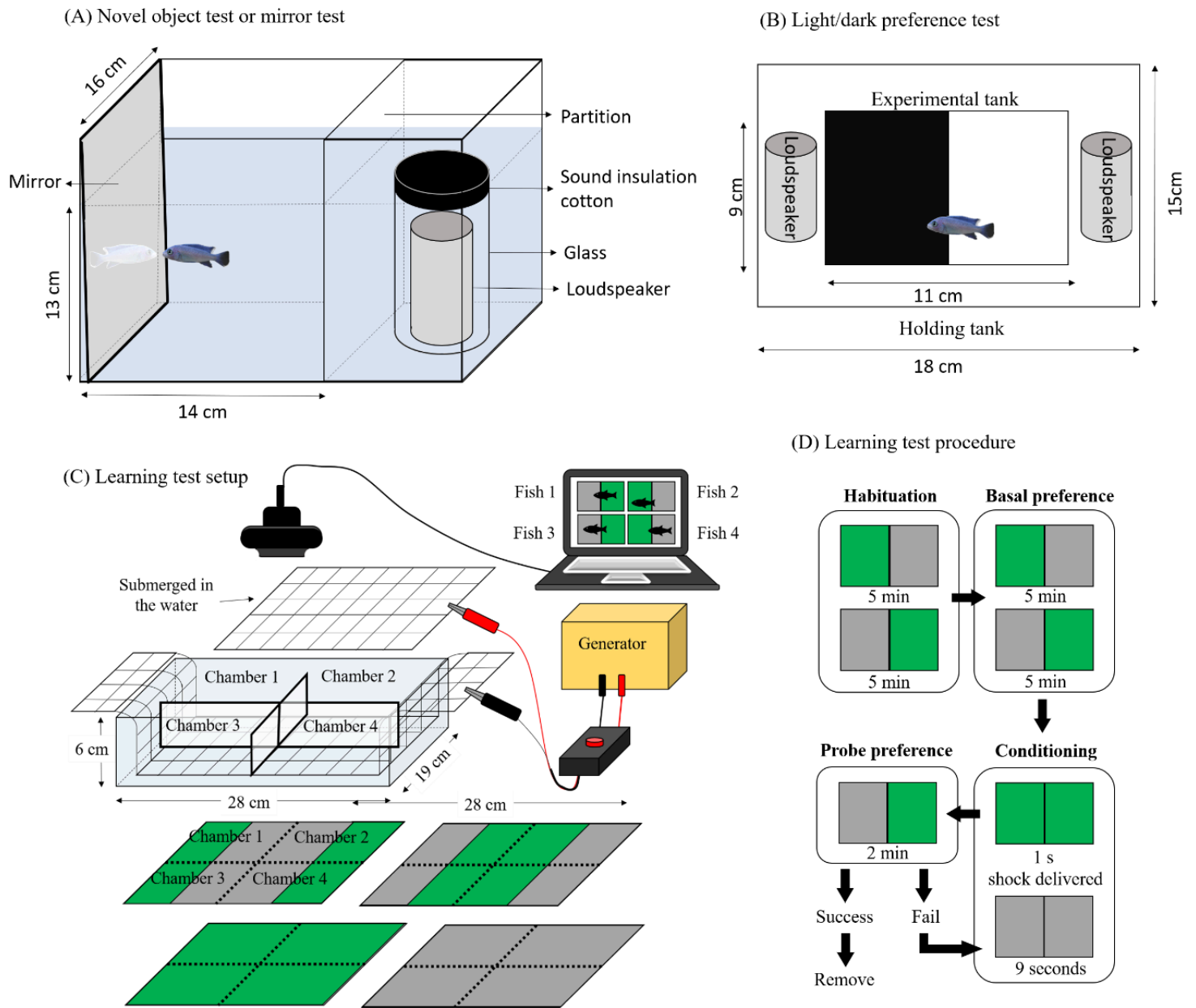


Figure 2

Experimental setup. (A) Front view of the novel object test and mirror test aquaria. The mirror was randomly placed on either the left or right side of the aquarium, with the left-side placement shown as an example in the figure. (B) Schematic top view of the Light Dark Preference Test arena. (C) Learning test setup. The transparent tank was divided into four chambers. The bottom of the tank was lined with a wire mesh, and another grid was submerged at the water surface. Both grids were connected to a generator using alligator clips to produce a closed electric field within the aquarium. Four coloured paper boards were prepared to serve as colour cues and placed on the bottom of the tank at different phases. Each board was the same size as the tank's base. The dashed line indicates the divider line of the chamber, which was not drawn on the board. (D) Schematic diagram of the learning test procedure in each chamber, showing the sequential phases conducted for each individual, including habituation, basal preference assessment, conditioning, and the final probe preference assessment

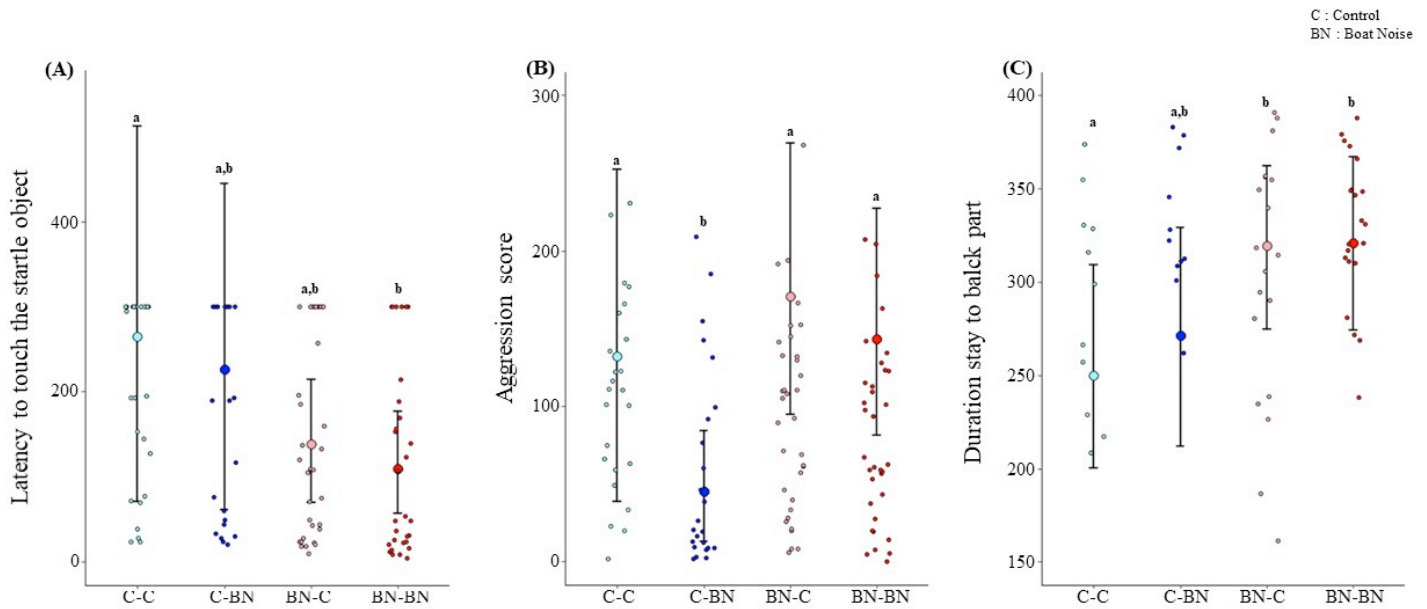


Figure 3

Model-adjusted means (posterior modes $\pm$ 95% credible intervals) from Bayesian GLMMs for behavioural responses across treatments. (A) Latency to touch the novel object (boldness); (B) Aggression score (mirror test); (C) Duration spent in the black compartment (anxiety-like behaviour). Experimental groups represent long-term treatments followed by testing conditions. C = control; BN = boat noise. Small circles represent individual observations. Different letters (a, b, or ab) indicate credible differences among treatments; groups sharing a letter are not credibly different, whereas groups with different letters are

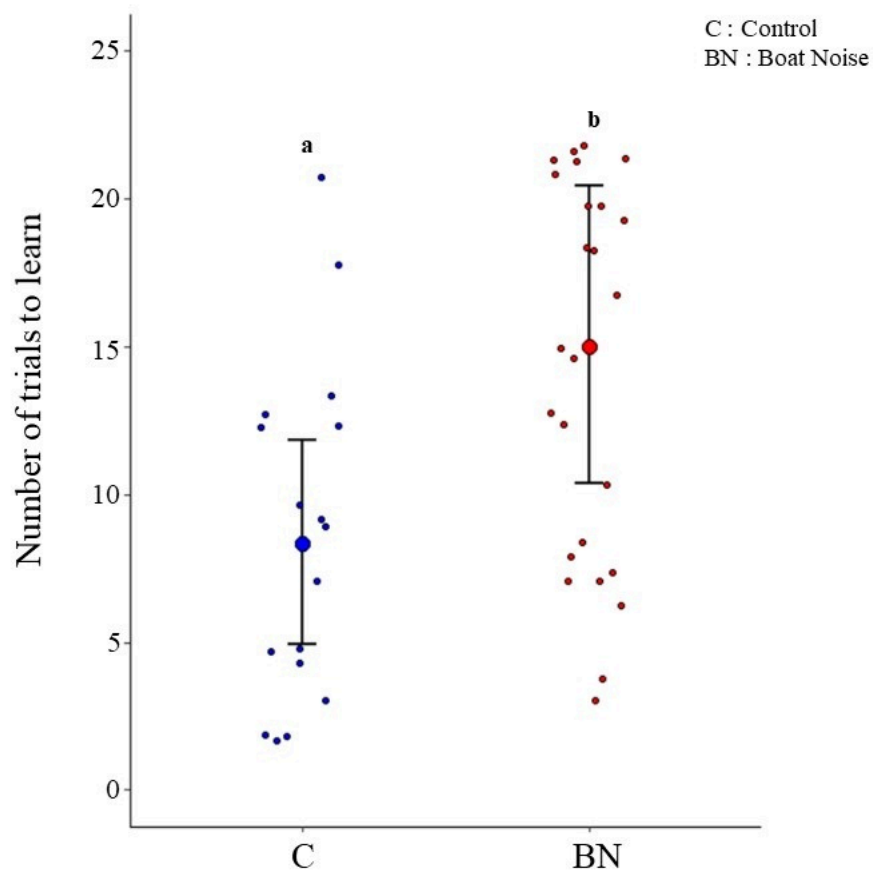


Figure 4

Model-adjusted means (posterior modes $\pm$ 95% credible intervals) from a Bayesian GLMM for the number of trials required to reach the learning criterion following long-term noise treatments. Different letters (a, b) indicate credible differences among treatments based on posterior credible intervals; groups sharing a letter are not credibly different, whereas groups with different letters are. C = control; BN = boat noise