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Effects of acoustic pollution on the sexual behaviour of male guppies (*Poecilia reticulata)*

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RESUMEN

La actividad humana es considerada una fuerza selectiva que actúa sobre los rasgos de otras especies. El ruido generado por el hombre se ha vuelto un elemento cada vez más invasivo en el ambiente, y el impacto de este contaminante involucra cambios fisiológicos, ecológicos y conductuales en los organismos. Dentro de estos últimos, los comportamientos reproductivos son particularmente relevantes porque están directamente relacionados al éxito reproductivo. En este trabajo, estudié cómo la contaminación acústica afecta el comportamiento sexual de un pez ovovivíparo, el guppy (*Poecilia reticulata*).

En el laboratorio, se utilizó ruido blanco intermitente para simular contaminación acústica y examinar sus efectos prolongados e inmediatos sobre el repertorio de comportamientos sexuales de los guppies macho. Para los efectos prolongados, comparé individuos expuestos durante cuatro días a un tratamiento de ruido o control y evalué su comportamiento antes y después de la exposición; para los efectos inmediatos, comparé el comportamiento de individuos expuestos en tiempo real a tratamientos de ruido y control. Medí la latencia para iniciar el comportamiento sexual, la frecuencia de balanceos del gonopodio e intentos de cópula forzada, la frecuencia y duración de despliegues sigmoides, así como hacia cuál hembra iban dirigidos estos comportamientos. A partir de los datos registrados, calculé el esfuerzo reproductivo (obtenido de la suma de intentos de cópulas forzadas y despliegues sigmoides ejecutados por un macho hacia ambas hembras), la elección de pareja (representada por la preferencia de una hembra en particular) y la tasa de estrategia de apareamiento usada por los machos focales (representada por la preferencia de una táctica reproductiva en particular).

Contrario a nuestras predicciones, no se observaron efectos inmediatos del ruido sobre ninguno de los rasgos evaluados. Sin embargo, tras una exposición de cuatro días a ruido, se observó un incremento en el esfuerzo reproductivo de los machos.

En conjunto, estos resultados indican que el comportamiento sexual de los machos de una especie dulceacuícola es influenciado por los efectos prolongados, pero no los inmediatos, de la contaminación acústica bajo condiciones de laboratorio. Con este proyecto se contribuye al cuerpo de literatura que demuestra que el ruido puede alterar el comportamiento, y se presenta evidencia de las potenciales perturbaciones que

puede ocasionar incluso en organismos que no se comunican acústicamente. Ciertamente, los futuros trabajos que evalúen los efectos de este contaminante en entornos acuáticos deberían considerar consecuencias concretas sobre la adecuación biológica para tener una imagen más completa del alcance de estos impactos.

ABSTRACT

Human activity is considered a selective force acting on other species' traits. Man-made noise has become an increasingly invasive element in the environment, and the impact of this pollutant involves physiological, ecological, and behavioural changes in organisms. Among the latter, reproductive behaviours are particularly relevant because they directly relate to fitness. Here, I studied how noise pollution affects the sexual behaviour of a livebearer fish, the guppy (*Poecilia reticulata*).

In the laboratory, intermittent white noise was used to simulate acoustic pollution and examine its long-term and immediate effects on the repertoire of sexual behaviours of male guppies. For long-term effects, I compared individuals exposed during four days to a noise or a control treatment and evaluated their behaviour before and after exposure; for immediate effects, I compared the behaviour of individuals during real-time exposure to noisy and control treatments. I measured the latency to initiate sexual behaviour, frequency of gonopodial swings and gonopodial thrusts, frequency and duration of sigmoid displays, and towards which female such behaviours were directed. From the recorded data, I calculated reproductive effort (obtained from the sum of gonopodial thrusts and sigmoid displays from a focal male towards both females), mate choice (represented by the preference of a particular female), and the rate of the mating strategy used by the focal males (represented by the preference of a particular reproductive tactic).

Contrary to our predictions, there were no immediate effects of noise on any of the assessed traits. However, after a four-day exposure to noise, an increase in the reproductive effort of males was observed.

Taken together, these results indicate that the sexual behaviour of males of a freshwater fish species is influenced by the long-term, but not the immediate, effects of acoustic pollution under laboratory conditions. We contribute to the body of literature

which shows that noise can alter behaviour, and we present evidence of the potential disturbances that it can introduce even in organisms that do not communicate acoustically. Certainly, future work on the characterisation of the effects of this pervasive pollutant on aquatic environments should consider concrete fitness consequences to get a full picture of the extent of these impacts.

INTRODUCTION

Besides climate change, invasive species introduction, overexploitation, and destruction of habitats caused by human interference, man-made pollution is one of the greatest threats against biodiversity, acting as one of the selective forces over species traits (Pelletier & Coltman, 2018). Anthropogenic pollutants can be perceptible and disturb organisms, but some others might manifest themselves subtly, indirectly, and unintentionally (Cross et al., 2021). Artificial noise and light, vibrations, and visual disturbances are some of the ‘enigmatic’ by-products generated by human activity. They were previously referred to as such due to their difficulty of being detected and quantified, and their mostly cryptic and accumulative effects (Cross et al., 2021).

Noise, as a pollutant produced by human intervention, occurs persistently in both aquatic and terrestrial environments (Kunc & Schmidt, 2019). Its accelerated expansion comprises significant effects even in isolated, not urban areas (Sordello et al., 2020). For this reason, it is extremely important to identify and evaluate the impacts that this low-profile, but pervasive contaminant has on the environment.

In several meta-analyses and reviews, it has been shown that noise affects a broad range of species belonging to different taxonomic groups (Kunc & Schmidt, 2019). Studies have been carried out on invertebrates (Classen-Rodríguez et al., 2021; Di Franco et al., 2020; Ferrier-Pagès et al., 2021; Gomes et al., 2022), fishes (Cox et al., 2018; Di Franco et al., 2020; Ferrier-Pagès et al., 2021; Mickle & Higgs, 2018; Popper & Hawkins, 2019; Slabbekoorn et al., 2010), amphibians (Gomes et al., 2022; Roca et al., 2016; Simmons & Narins, 2018), reptiles (Simmons & Narins, 2018), birds (Gomes et al., 2022; Halfwerk et al., 2018; Potvin, 2017; Roca et al., 2016), and mammals (Bednarz, 2021; Erbe et al., 2019; Halliday et al., 2020; Nabi et al., 2018).

Since freshwater ecosystems hold an incredible amount of biological richness, and at the same time are a constant target of human exploitation, they are viewed as hotspots of endangerment (Reid et al., 2019). Noise may be introduced to these habitats by terrestrial or aerial sources (Rountree et al., 2020), including industrial and commercial activities such as: shipping transportation, underwater resource extraction, seismic exploration, urban development and construction, and recreational activities (Bolgan et al., 2016; Popper & Hawkins, 2019). It might be done intentionally for specific purposes (e.g., seismic surveys), or unintentionally as a by-product of other human activities (Slabbekoorn et al., 2010).

Artificial noise can influence the physical characteristics of the environment, and therefore affect the quantity and quality of the information that organisms can obtain from it (Candolin & Wong, 2019). Consequently, plastic changes, local adaptations, relocation, and/or extinction of populations of wild organisms are expected as a response to noise (Pelletier & Coltman, 2018). General effects have been documented on behaviour, physiology, communication, reproduction, development and growth, space use, interspecific relationships, migration, foraging, learning and memory in animals (see for example: Gordon & Uetz, 2012; McLaughlin & Kunc, 2013; Osbrink et al., 2021; Park & Do, 2022; Wisniewska et al., 2018).

Here, I examined how white noise influences the repertoire of sexual behaviours of the Trinidadian guppy (*Poecilia reticulata,* Peters), a small tropical livebearer, on a relatively long-term basis and during a one-term exposure to the noise. I carried out this by comparing the latency to initiate sexual behaviour, frequency of gonopodial swings and thrusts, frequency and duration of sigmoid displays, reproductive effort, mate choice, and mating strategy between males exposed to noise and a control group.

BACKGROUND

Generalities of sound and its measurement

Sound is defined as the propagation of waves through an elastic medium (e.g., air, water), generated by the vibration of an object (Charif et al., 2010). Since water is a denser medium, sound travels about 4.8 times faster and much greater distances within it than in air (Popper & Hawkins, 2019). These travelling waves can be detected as fluctuations in the pressure within the medium, caused by the compression and rarefaction of particles, which is known as sound pressure (scalar measurement, i.e., consists of magnitude) (Nedelec et al., 2016a; Popper & Hawkins, 2019). Sound is also accompanied by particle motion (vector measurement, i.e., consists of magnitude and direction), defined as the oscillation of particles and its neighbours in response to a vibrating source (Nedelec et al., 2016a; Popper & Hawkins, 2019).

Sound pressure is usually expressed in pascals (Pa) or micropascals (µPa), which can be obtained by using a piezoelectric sensing device that responds to pressure fluctuations in the water: a hydrophone (Martin et al., 2016). Numerous software have been developed to carry out a more profound acoustic analysis. On the other hand, particle motion can be measured in terms of displacement (m), velocity (m/s), or acceleration (m/s^2), using different methods and instruments, like hydrophones, geophones, or accelerometers (Martin et al., 2016; Popper & Hawkins, 2019). The last one is more appropriate for acoustic measurements (see Nedelec et al., 2016a). An accelerometer measures the relative motion between its body and a denser medium; it functions by transducing changes in acceleration into current fluctuations, which are digitally recorded as voltage fluctuations (Nedelec et al., 2016a).

During sound propagation, the signal may suffer from several changes due to absorption, reverberation, or attenuation in the medium (Bjørnø & Buckingham, 2017). In some particular cases (e.g., under acoustic free-field conditions), particle motion can be calculated from sound pressure, but since most of the time there exists a complex relationship between both components (e.g., in shallow waters where acoustic boundaries are present), it is recommended that each of them should be measured directly (Nedelec et al., 2016a; Popper & Hawkins, 2018).

When conducting research involving sound measurement, it is considered that studies should be conducted ideally in the field to minimise distortions due to reverberation, which occurs in enclosed spaces as a result of multiple reflections (Akamatsu et al., 2002). It is also argued that open bodies of water are a more realistic approach to natural conditions. However, many challenges must be tackled, for example: individuals are hard to identify and follow, as well as to obtain physiological and corporal condition measurements from them; it is also difficult to control or measure environmental conditions and identify other influencing factors; and lastly, natural background noise and its spatial and temporal fluctuations should be considered (Slabbekoorn, 2016).

On the other hand, indoor studies are more practical, feasible, cheaper, less time consuming, and they also possess the unique capacity of enabling the standardisation of conditions. The acoustic validity of indoor studies can be more limited (Akamatsu et al., 2002; Slabbekoorn, 2016), and there is an ongoing debate in whether this kind of studies would likely match conditions found in aquatic natural environments. However, it was recently revealed that results given by laboratory experiments are as valid as the ones given by field experiments and could even be applied to broader community effects (Pieniazek et al., 2020). Furthermore, it is worth noting that fishes and other aquatic organisms might be adapted to detect sound under a wider variety of conditions since they have not evolved in a quiet environment (Cox et al., 2018; Radford et al., 2014); therefore, although the acoustic conditions may not match the natural ones, we can expect that individuals will still be able to respond to them.

The effects of sound and noise on aquatic life

Sound itself does not represent a problem since most organisms use this resource to successfully carry out many of their activities (e.g., communication with conspecifics, reproduction, predator alerts, defence mechanisms) (Slabbekoorn, 2016; Sordello et al., 2020). However, sound turns into noise when it becomes unpleasant or disruptive, contains little or no information, or overcomes the auditory sensitivity threshold of an animal, bringing permanent or temporal negative repercussions (Cox et al., 2018; Popper & Hawkins, 2019; Sordello et al., 2020).

The acoustic scene is an exceptionally important information source for perhaps most aquatic animals, especially because of its flexible detection and its slow attenuation in comparison with other cues (Popper & Hawkins, 2019). The natural underwater soundscape comprises a mixture of geophysical and biological sounds, while sounds emitted by human activities introduce noise (Nedelec et al., 2021).

Noise can have differential impacts on species depending on their sensory capacities (Slabbekoorn, 2016). Hearing is found in all fishes, thanks to both an inner ear and the lateral line system. They are typically sensitive to low-frequency sounds, presumably most of them responding to a 100-400 Hz range (Popper, 2003; Slabbekoorn, 2016), although differences in the sensory capacities between species are ubiquitous. The auditory detection continuum is a scale that represents the variation in sensitivity and spectral range of fish hearing, and it is based on the presence or absence of specially evolved morphological structures, such as the Weberian ossicles present in members of the superorder Ostariophysan, which enables detection of frequencies up to 4000 Hz (Popper & Fay, 2011; Slabbekoorn et al., 2010).

The impact of noise may be attributed to the particle motion or the pressure component of sound, or a combination of both (Rogers et al., 2016; Slabbekoorn, 2016). Even though most studies have focused on sound pressure only, many fishes are not able to directly sense this component as this depends on the presence of air-filled cavities (e.g., swim bladder) which act as acoustic transformers (or a resonating chamber) (Nedelec et al., 2016a; Popper & Hawkins, 2018, 2019; Rogers et al., 2016). In fact, all fishes can detect and respond to particle acceleration rather than to sound pressure, because their inner ear closely resembles an accelerometer-like system: a mass (the otolith or otoconia) that moves in a relative manner to some kind of receptor (sensory hair cells) (Popper & Fay, 2011; Popper & Hawkins, 2018; Rogers et al., 2016).

Examining changes in behaviour is a popular method used to identify the state of an animal's well-being (Mickle & Higgs, 2018). Animal behaviour assays are widely used as indicators of pollution, serving as early warnings of stressful conditions thanks to its easy quantification and sensitivity (Blocker & Ophir, 2013; Francis & Barber, 2013). Since behavioural responses to sound are influenced by cognitive processes (e.g., detection, classification, decision-making), they can be highly compromised by any disturbance in the environment (Mickle & Higgs, 2018; Slabbekoorn et al., 2010).

The literature clearly demonstrates that when exposed to noise pollution animals can respond in several ways, and its effects oscillate from being not deleterious at all to potentially dangerous (Masud et al., 2020; Potvin, 2017). Changes in feeding, mating, spawning, orientation and navigation, habitat selection, stress levels, hearing thresholds, capacity of detection of biologically relevant sounds, and communication (Popper, 2003; Popper & Hawkins, 2019; Potvin, 2017) are expected. High levels of noise may also cause death or mortal injuries to fishes, but even at lower levels it can cause significant harm (Popper & Hawkins, 2019).

Sounds of differing characteristics (e.g., frequency range, intensity, and duration) have different effects (Popper & Hawkins, 2018). For example, impulsive or acute sounds (i.e., transient and brief), combined with high frequency, are usually more linked to create disturbances within the aquatic environment; nevertheless, low-amplitude continuous or chronic sounds (i.e., of long duration) are much more widespread and have deleterious impacts (Colbert, 2020). Low frequencies (<500 Hz) represent most of the dominant frequencies of underwater noise generated by human activity and often overlap the acoustic signals of animals (Mickle & Higgs, 2018; Slabbekoorn, 2016). However, many studies have focused on high-frequency noises sources such as pile driving, sonar, and airguns (Mickle & Higgs, 2018).

Several studies have reported that anthropogenic noise negatively affects individual (e.g., shelter maintenance, antipredator defence) and collective behaviours (e.g., aggressive and submissive behaviour between dominant pairs and their subordinates) (see for example: Bruintjes & Radford, 2013; Butler & Maruska, 2020; Short et al., 2020). Furthermore, behavioural responses to noise include changes in temporal patterns, spatial distributions or movements, foraging or provisioning efficiency, vigilance behaviour, territorial defence, and mate attraction (Francis & Barber, 2013).

Extreme overexposure to noise is often associated with physical damage, while more moderate exposure is linked to subtle effects through behavioural perturbations; nevertheless, these last effects could also reflect a high exposure to noise but act as a delayed effect (Slabbekoorn, 2016). Changes in behaviour caused by noise exposure could have consequences not only at an individual level, but also could generate ecological variations in the long term and have an impact on the population's viability and species survival (Candolin & Wong, 2019). Furthermore, disruption of signal detections can occur not only at intraspecific but also at interspecific level (Francis & Barber, 2013).

Freshwater ecosystems are naturally noisy, but anything that hampers the biologically relevant signal flow will have a potentially deleterious effect on organisms (Popper, 2003). For instance, the blacktail shiner (*Cyprinella venusta*) exhibits a significant elevation in cortisol levels and hearing thresholds immediately after exposure to road traffic noise (Crovo et al., 2015). Groups of European minnows (*Phoxinus phoxinus*), a fish that shows collective behaviour, change their shoal distribution and social dynamics when exposed to different artificial signals consisting of tones and Gaussian white noise (Currie et al., 2021). In the presence of low-frequency boat sounds, the lake sturgeon (*Acipenser fulvescens*) decreases its maximum calling rate, suggesting that intraspecific communication during spawning might be interrupted during commercial shipping activity (Higgs & Beach, 2021).

Members of the Families Cyprinidae, Centrarchidae, and Percidae are the most studied regarding noise interference in freshwater ecosystems (e.g., *C. venusta*, *Danio rerio*, *Lepomis macrochirus*) (Zavala-Rodríguez, unpublished data; Figure 1). Interestingly, effects over non-soniferous freshwater fishes remain still quite unexplored. This gap in our understanding underscores the critical need for comprehensive studies on the effects of noise on non-soniferous freshwater fishes, opening new avenues for research that could significantly enhance our grasp of aquatic ecosystem's acoustic dynamics.

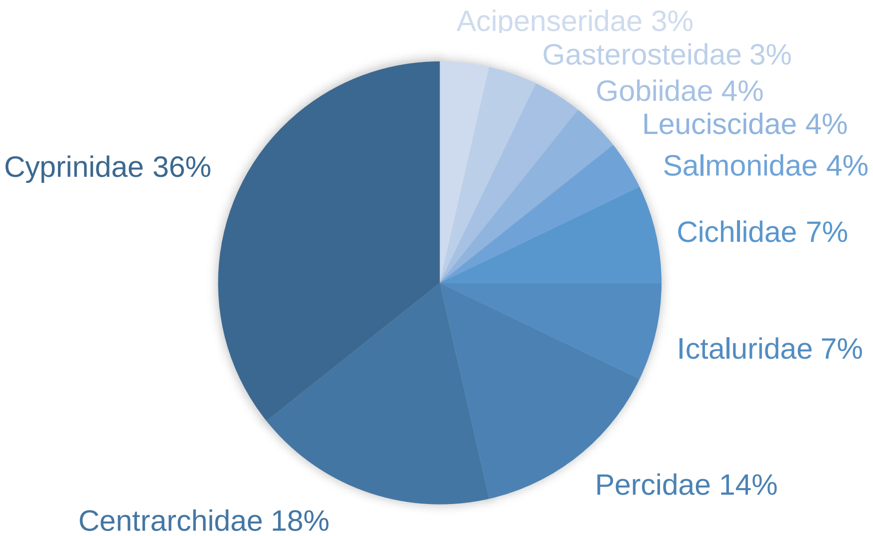

Figure 1. Most studied fish families in relation to the effects of noise pollution.

Precopulatory sexual selection under environmental constraints

Sexual selection is usually described as the consequence arising from differential reproductive success caused by competition for access to mates or fertilisation opportunities (Jones & Ratterman, 2009). Since sexual selection is directly related to fitness, it strongly affects offspring production, survival, and population dynamics (Jones & Reynolds, 1997).

Precopulatory elements taking part in sexual selection include ornaments and behaviours such as courtship displays, which highlight individual quality and indicate the potential benefits, direct and indirect, towards the opposite sex (Andersson, 1994). This increases the possibility of obtaining a partner and facilitates mating (Candolin & Wong, 2019; Mitoyen et al., 2019). In general, when traditional sex roles are observed, males tend to exhibit these behaviours, which include olfactory, auditive, and visual signals, predominantly conspicuous dances and acoustic calls (Blocker & Ophir, 2013; Mitoyen et al., 2019); nevertheless, females are not exempt from carrying out courtship rituals. Mate choice is also an essential element of sexual selection. Mating preferences leads to the individuals with the most preferred traits having greater numbers of offspring and increasing the frequency of these traits within the population (Jones & Ratterman, 2009). Through an animal's behaviour it is possible to identify these preferences.

Since sexual behaviours are particularly sensitive (Jones & Reynolds, 1997), they are considered as condition-dependent indicators (Andersson & Simmons, 2006). Stressful conditions triggered by anthropogenic pressures can cause clear changes in these behaviours in several taxa (Table 1) and affect, usually in a negative way, the strength of sexual selection (Heinen-Kay et al., 2021).

Table 1. Impact of noise pollution on male sexual behaviours across some species.

Species	Methods	Findings	Reference
Mediterranean field cricket (*Gryllus bimaculatus*)	Exposure to traffic and white noise treatments.	Reduction and disturbance of male signal production after exposure to white noise.	Bent et al., 2021a
Snouted tree frog (*Scinax nasicus*)	Recording and comparison of vocalisations in areas with and without traffic noise.	Shifts in the temporal and acoustic properties of call structure in traffic noisy ponds.	Leon et al., 2019
Painted Goby (*Pomatoschistus pictus*)	Exposure to a low-frequency noise treatment.	Shifts in the use of acoustic and visual signalling modalities for	de Jong et al., 2018b

		spawning decisions in females.	
Skylark (*Alauda arvensis*)	Recording and comparison of vocalisations in areas with and without wind farms noise.	Frequency shift in the song of males closer to wind turbines.	Szymański et al., 2017
Humpback whale (*Megaptera novaeangliae*)	Field exposure to low-frequency active sonar.	Increase of song duration during sonar playbacks.	Miller et al., 2000
Grey-headed flying-fox (*Pteropus poliocephalus*)	Recording and comparison of vocalisations in an area with an urban noise gradient.	Cessation of courtship vocalisations (*silentium effect*) when aircraft fly-overs were present.	Pearson & Clarke, 2018

Poecilia reticulata **as a model study system**

Trinidadian guppies belong to the cyprinodont family Poeciliidae, which is characterised by internal fertilisation and giving birth to live offspring (Houde, 1997). These poeciliids are native to Trinidad y Tobago and adjacent parts of South America, but nowadays they are found in many countries where they had been introduced with the aim of controlling mosquito plagues (Houde, 1997). They usually occur in small, clear, and relatively sterile streams in mountain forest areas, but also in larger and more turbid lowland streams (Houde, 1997).

This species shows an extreme degree of sexual dimorphism, colour patterns, and courtship displays (Houde, 1997). Male guppies are highly polymorphic and may possess one to several colour patches (orange, yellow, iridescent blue or green, red, pink) and black spots (Liley, 1966) (Figure 2). The anal fin is modified (three rays are thickened and elongated) into an intromittent organ named the gonopodium, which allows the transference of sperm into the female (Houde, 1997; Liley, 1966).

Figure 2. Female and male wild Trinidadian guppies under laboratory conditions. The male positioned himself behind the female (right side of the picture). Picture taken by X. Zavala-Rodríguez.

Guppies possess a mating system broadly termed promiscuous, in which males invest heavily in sexual behaviours in terms of time, energy, and risk (Magurran & Seghers, 1994). As in many highly competitive sexual environments, their mating strategy includes two alternative tactics: 'forced copulation attempts' and 'courtships' (Figure 3), the frequency of which may be plastic (Auld et al., 2015). A detailed description of these behaviours is given in Liley (1966) and Baerends et al. (1955).

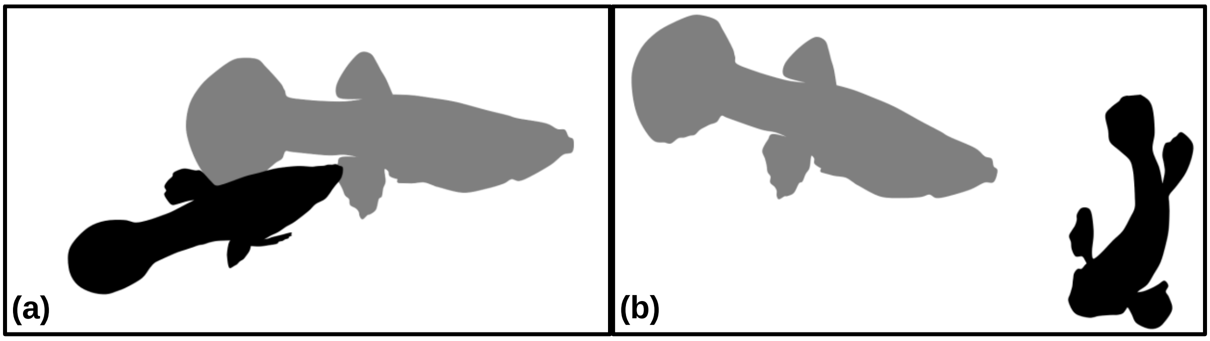

Figure 3. Schematic representation of male mating tactics in guppies. Females are in grey and males in black. (a) *Forced copulation attempt.* Attempt to introduce the male's gonopodium in the genital opening of the female, also known as gonopodial thrust. (b) *Courtship.* Performance of a courtship behaviour known as sigmoid display, to obtain a 'true' copulation.

The guppy mating system has been widely exploited in research, playing a major role in studies that intend to elucidate how stressors impact organisms. Most of these studies evaluate the effect of chemical pollutants on male sexual behaviour, a research field known as behavioural ecotoxicology. One outstanding group comprises the endocrine disrupting chemicals (EDCs), which have been found not only to affect the natural development and reproduction of organisms, but also to have the potential to generate changes in sexual selection (Bertram et al., 2015). Research includes environmentally realistic exposure to: 17β-trenbolone (Bertram et al., 2015), the alkylphenol 4-*tert*-octylphenol, capable of mimicking the action of 17β-estradiol (Bayley et al., 1999), or the herbicide atrazine (Shenoy, 2012).

Currently, little is known about the hearing abilities of guppies, other than that they only possess the ‘basic’ auditory morphology of all fishes (Martin et al., 2016). The only known study that demonstrates that guppies use auditory cues has been performed by De Waele et al. (2022), who observed that the sound of moving water is employed to orient their jumping. There is a single study known to have investigated the effect of noise exposure on guppies, in which was found that fish experiencing acute noise increased their susceptibility to disease, and those exposed to chronic noise had a lower parasite burden but were more prone to die earlier (Masud et al., 2020).

Even though there does not exist an audiogram of *P. reticulata*, there are some available studies from closely related species. For example, the Atlantic molly (*Poecilia mexicana)* hearing abilities have been described by Schulz-Mirbach et al. (2010). This species has no hearing specialisations; therefore, they primarily detect the particle component of sound. By applying the auditory evoked potential (AEP) technique, it was found that the Atlantic molly was more sensitive at 200 and 300 Hz, having the auditory thresholds abruptly increased at higher frequencies than 300 Hz; but fish responded to frequencies of up to 1500 Hz, of course needing higher sound levels (dB) to do so (Schulz-Mirbach et al., 2010). Particle acceleration level audiograms displayed lowest thresholds at 200 and 300 Hz. This study also showed that this species does not communicate acoustically since there was no evidence for sound production. Based on the above, we estimated the upper and lower frequency limits of the hearing range of Trinidadian guppies (100-1500 Hz).

OBJECTIVES

General objective

To investigate the long-term and immediate effects of artificial white noise on the sexual behaviour of male guppies.

Specific objectives

- To compare the latency to start displaying any sexual behaviour between males exposed to noise and control males exposed to ambient noise.
- To compare the frequency of gonopodial swings, thrusts, and sigmoid displays, and the duration of the last one, between males exposed to noise and control males exposed to ambient noise.
- To compare the reproductive effort, the mate choice and the rate of the mating strategy used between males exposed to noise and control males exposed to ambient noise.
- To determine whether morphometric traits (weight and length) and colouration influence any of the behavioural traits assessed.

HYPOTHESIS AND PREDICTIONS

Artificial noise can cause disturbances in animal behaviour. Male guppies' sexual behaviour is very sensitive to environmental conditions and has proven to be a great indicator of the presence of pollutants. Consequently, white noise would have a long-term and immediate negative effect on the sexual behaviour of male guppies. It was predicted that after being exposed to a noise treatment and during a one-term exposure:

- Males would increase their latency to initiate displaying sexual behaviours, perform less sexual behaviours (gonopodial swings, gonopodial thrusts, and sigmoid displays) and spend less time courting than those exposed to control conditions, hence they would show a decrease in their reproductive effort.

- Males would not exhibit clear preferences for a specific female in a pair, nor would they preferentially adopt a particular mating tactic; therefore, their mate choice and mating strategy would differ from those exposed to control conditions, who would show a marked preference for the bigger female and for one mating tactic.
- Males exhibiting greater body weight, length, and more extensive orange body colouration would show a reduced stress response, suggesting that males with enhanced physical and colouration attributes would be less impacted by noise.

JUSTIFICATION

The constant expansion of human activity in aquatic environments has brought the need for studies that evaluate how organisms deal with the changes introduced. Marine ecosystems had received most of the attention with this regard, leaving freshwater species quite understudied (Strayer & Dudgeon, 2010). However, freshwater ecosystems face intense anthropogenic pressures and threats that will likely be stronger in the future, especially lotic habitats such as rivers, streams, marshes, and lakes. In fact, it has been revealed that there is an ongoing rapid population decline and many species inhabiting these sites are prone to extinction risk, in comparison with terrestrial organisms (Collen et al., 2014; Reid et al., 2019). The present study will contribute to an increasing body of knowledge about the effects of noise pollution on aquatic life. We can also get an insight of the importance of the establishment of policies, regulations, and joint efforts that have the aim of reducing man-made underwater noise and mitigate its effects.

Furthermore, noise impact has only been tested in a few species, especially in those that communicate acoustically. However, although it might be argued that these animals are the ones most affected by anthropogenic sound, almost all species obtain a great amount of information about their environment through their soundscape. Indeed, it is very likely that hearing in most animals has evolved to broaden the capacity of detecting the constant flow of information present in the environment, not for acoustic communication *per se* (Popper, 2003). Using a non-soniferous species in this study will help to broaden this perspective and thus contribute to a better understanding of the impacts of noise pollution.

Additionally, noise may have implications for animal welfare. Guppies and other poeciliids are commonly used as model species in laboratory research and are popular ornamental organisms in aquaculture. For housing, they are usually placed in tanks equipped with noisy water filters and air pumps, which could serve as significant stressors. As this noise resembles white noise, comprehending its effects on these organisms is crucial for encouraging the innovation of alternative aquarium equipment, thus promoting the well-being of captive animals.

Finally, since the mating system of Trinidadian guppies is widely recognised as a valuable model in sexual selection theory, our results will also strengthen the theoretical framework on anthropogenic factors shaping this evolutionary force.

MATERIALS AND METHODS

I conducted two independent experiments where male guppies were subjected to the same type of noise, yet the exposure method varied significantly: one involved a 4-day noise exposure prior to the behavioural test (long-term effect experiment), while the other entailed no prior exposure, with noise introduced exclusively during the test itself (immediate effects experiment).

Collection and housing

Wild Trinidadian guppies were collected on February 28th at 10 a.m. at a small lake located in La Magdalena, Tepeojuma, Puebla, México (grid reference 18.70652° N, 98.43910° W) (Figure 4). This lake was situated approximately 2-3 km away from a moderately busy highway, and the driveway was located at a distance of 3 m. Also, a few metres away there was a well (probably used for wastewater treatment) which emitted a constant but very subtle noise. The fish were transported in coolers by car to the Laboratory of Evolutionary Ecology of the Faculty of Biological Sciences at the Benemérita Universidad Autónoma de Puebla.

Figure 4. Lake where collection was conducted. Picture taken by X. Zavala-Rodríguez.

Guppies were kept under laboratory conditions in 80-250 L tanks (2 fish per L), each of them equipped with an automatic heater, two internal filters containing activated carbon and bio-spheres, artificial plants, and approximately 4 cm of gravel. The tanks were maintained at 26 ± 1.5 °C and a pH level of 7.5. Fish were submitted to a 12:12 light:dark cycle and were fed twice a day with *TetraMin*® Tropical Flakes *ad libitum* for 1 month since their arrival for acclimatisation. Water quality levels (pH, nitrite, nitrate, and ammonium) were weekly monitored using an *API*® Freshwater Master Test Kit.

Experimental fish

All experimental individuals were randomly selected from the stock tanks. Focal males were sexually mature and displayed sexual behaviours; we verified this by checking that they showed clear colour patterns and complete gonopodium development (Houde, 1997). Mature, non-virgin females (those housed in the stock tanks with males) were selected as the stimulus fish in the trials. Female guppies have a small sexual receptivity window which occurs once they have reached sexual maturity and remain virgins, or within a few days of giving birth to a litter of young; but they are unresponsive and appear to simply ignore males' displays at any other time (Houde, 1997). The latter mostly occurs when living in close contact with males, since it is assumed that they are either pregnant or inseminated (Liley, 1966). Therefore, its response behaviour toward every male is expected to remain constant.

Once the experiment was over, all fish were returned to a separate stock tank, and were not used in future trials. Thus, focal males in both experiments were not the same.

Experimental design for long-term effects

To test any long-term effects of acoustic pollution on the sexual behaviour of male guppies, focal males were randomly assigned to two treatments in which the fish were exposed to: (1) control (ambient noise; represented by internal and external noises that could not be eliminated), and (2) noise treatment (white noise for 12 hours each day, see below). Behavioural trials were conducted before (hereafter Be) and after (hereafter Af) the exposure to treatment for each focal male (Figure 5).

The experiment was divided into four stages: acclimatisation period (3 days), behavioural trials (Be; 30 minutes each), treatment period (4 days), behavioural trials (Af; 30 minutes each) (Figure 5). Importantly, this experiment was conducted in conjunction with a sperm quality study (López-Flores, 2024); therefore, after the behavioural trials each focal male was stripped to collect the spermatophores expelled.

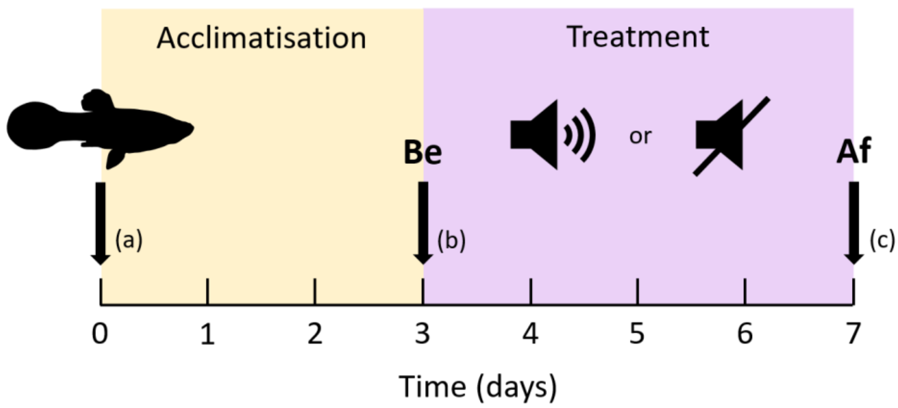

Figure 5. Simplified schematic representation of the stages that each batch of fish underwent in the long-term effects experiment. (a) Aleatory selection of four focal males and introduction to the acclimatisation tank. Before the acclimatisation period of three days (peach colour in the scheme), length and ID was registered for each male. (b) First behavioural trials (Be) were performed. Weight and picture of each individual was obtained before they were introduced randomly to a treatment tank. Treatment period of four days

(purple colour in the scheme) consisted of either exposure to noisy or control conditions. (c) Second behavioural trials (Af) were performed (~12 h after the treatment ceased).

Experiments were conducted from June to August 2023. We tested 23 males exposed to the noise treatment and 27 males to the control treatment. During behavioural trials, the observer was blind to the fish's identity and its treatment. It was not feasible to test all the individuals the same day; consequently, thirteen batches, each of them consisting of 4 males (excluding the last one, consisting of 5 males), were established. Because some males (n = 3) died during the experiment, 50 males were tested in total.

In order to get a realistic approach of a shallow-water system, which is a natural habitat of many freshwater fishes, four 40 L circular tanks with a diameter larger than its depth (60 cm diameter x 30 cm height) were used (Akamatsu et al., 2002). Acclimatisation tanks (n = 2) were equipped with an automatic heater, an internal filter, approximately 1 cm of gravel, and 4 individual square containers accommodated equidistant between them. Treatment tanks (n = 2) were identical to those used for acclimatisation, but included a speaker suspended in the centre (Figure 6). The speaker placed in the control tank was switched on but without sound playback, the latter to discard any possible electrical interference and to control for potential electrical radiation.

In addition to the focal males, two females per male were placed in each tank, which swam freely throughout it. This was done with the aim of stimulating visually and chemically, both the sexual activity and sperm production of males, a response known as priming (Bozynski & Liley, 2003).

Individual square transparent containers were used to confine individual fish (focal males) and guarantee an equal and stable exposure to noise. Two of their sides (the lateral ones) were covered with black contact paper to provide visual isolation between males, but not with females (front and back) (Liley, 1966) (Figure 6). The latter because it has been observed that the presence and behaviour of conspecific males also affects male sexual behaviour (Auld et al., 2015; Órfão et al., 2019).

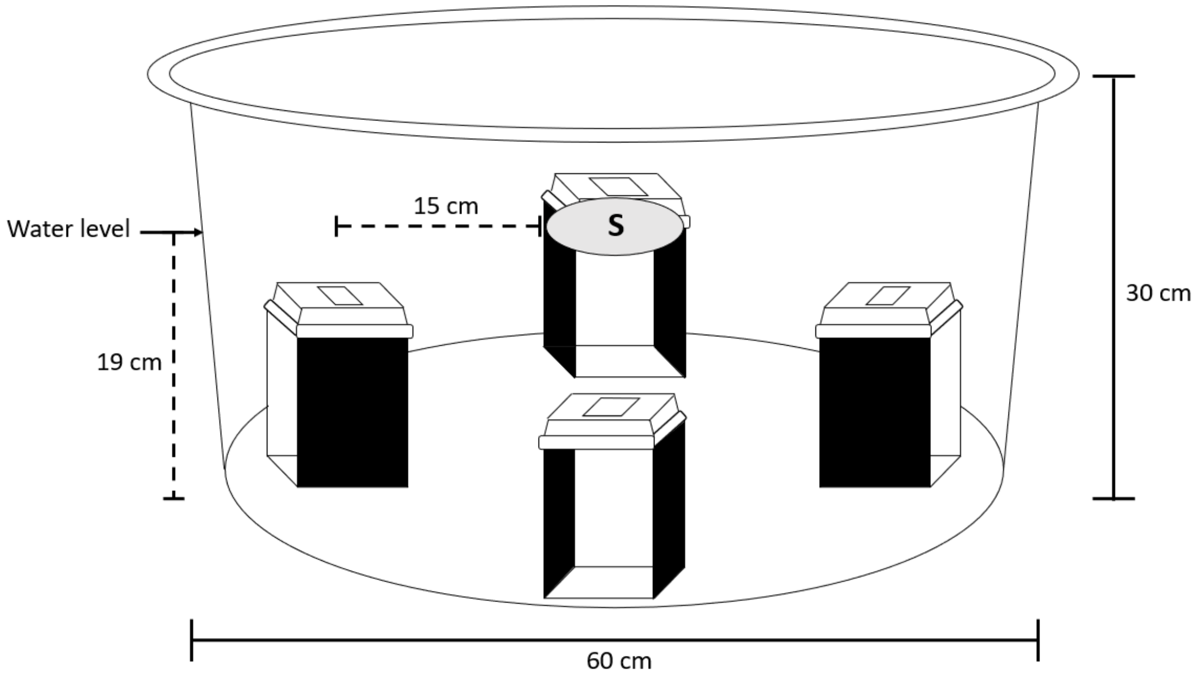

Figure 6. Experimental tank settings. A round tank contained four small cubical individual containers that housed each focal male. Dark fillings at 2 sides of each individual container represent the black contact paper covering the lateral view. The speaker (S) is suspended in the centre of the tank just above the water filter (the latter was not illustrated) and 15 cm away from each individual container.

Males were randomly placed in one of the treatment tanks. During this period, fish were maintained under the same temperature, light, and water quality levels to which they were exposed from the time they entered the laboratory. Each male was individually fed with approximately 1 mg of tropical flakes, while females were given a small pinch. Tanks were only handled during feeding, when changing reloaded speakers, and when 3 fish carcasses were removed. To minimise external noise and vibrations reaching the control tank and to escape the noise treatment tank, the bottom of both tanks was equipped with cardboard and thick foam. The top was also covered with an opaque material to prevent any external disturbance reaching the tanks. In addition, to maintain the quality of the water in the tanks, water filters remained on during both treatments.

Experimental design for immediate effects

To test any immediate effects of acoustic pollution on the sexual behaviour of male guppies, focal males were placed inside an observation tank with two females (see Behavioural trials; Figure 8) which included a speaker suspended on the surface, in the centre of the tank. The speaker always remained switched on, but sound playback was controlled according to the treatment; and fish could be located from 0 to 15 cm away from the speaker. Each male's behaviour was evaluated during 30 min while exposed to both noisy and control treatments, on consecutive days.

The experiment was divided into three stages: acclimatisation period, first behavioural trial (hereafter T1), second behavioural trial (hereafter T2) (Figure 7).

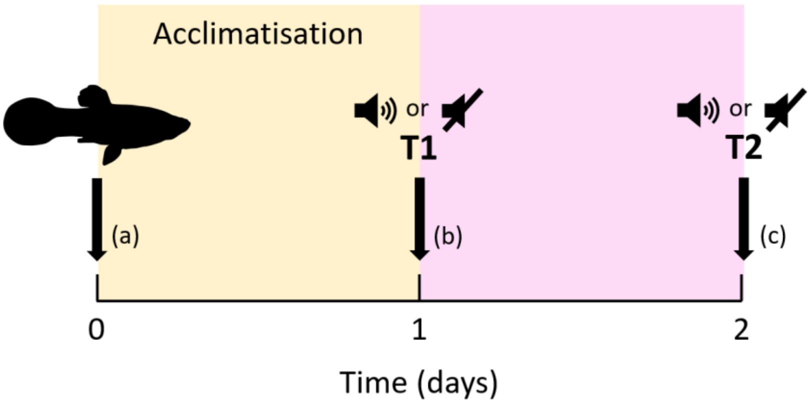

Figure 7. Simplified schematic representation of the stages that each batch of fish underwent in the immediate effects experiment. (a) Aleatory selection of four focal males and introduction to the acclimatisation tank (same design as in the long-term effects experiment) (peach colour in the scheme), where they stayed ~24 h. (b) First behavioural trial (T1) was performed. It was randomly established whether the male underwent the noise or the control treatment first. (c) Second behavioural trial (T2) was performed. Here, the male underwent the opposite treatment to T1.

Experiments were conducted from January to February 2024. 26 males were tested, but since it was not feasible to test all the individuals the same day, seven batches were established (6 of them consisting of 4 males, and one of 2 males).

Behavioural trials

Free-swimming trials were performed to evaluate male's sexual behaviour. For this purpose, each male was individually transferred into a rectangular observation tank, which was slightly illuminated with a common desk lamp and had 3 of its 4 sides covered with black foam to provide a background that allowed all movements to be appreciated (Liley, 1966) (Figure 8). All trials were conducted between 08:00 and 12:00 h, when guppies are more sexually active (Houde, 1997). Observations were made by a single observer (X. Zavala-Rodríguez).

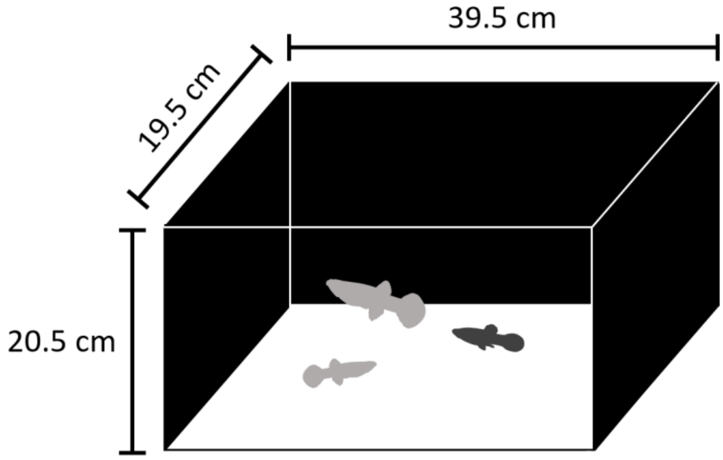

Figure 8. Observation tank settings.

Each trial consisted of a focal male and two females placed in the observation tank for 30 minutes. The pair of females consisted of a small one (same body length as the male) and a large one (5 ± 1 mm larger than the male), both coming from the same stock tank of the focal male. Focal males were used only once, but females participated several times in other batches, never with the same female partner nor the same day.

Prior to start observations, the pair of females to be tested were introduced in the observation tank 10 min before the male. As soon as the male was introduced, the trial started. I recorded the data in real time by sitting quietly behind a black curtain with a 15.5 x 3 cm hole in front of the tank, which prevented the individuals from being aware of my presence. I recorded the focal male behaviours defined in Table 2 using the software BORIS v. 8.9.19 (Behavioural Observation Research Interactive Software; Friard & Gamba, 2016).

Table 2. Description on male guppies' sexual behaviours

Behaviour	Definition	References
Latency	Time until the first sexual behaviour is displayed.	-
Gonopodial swing	To one side and forward movement of the gonopodium. Its extreme form includes the spread of the dorsal fin and the arching of the body horizontally. Increases in frequency of this movement is related to an increase of sexual activity, but it can be performed even when the female is not near. However, this behaviour is not directly associated with one of the mating tactics.	(Baerends, 1955; Liley, 1966).

Gonopodial thrust	Used to achieve a forced copulation without female cooperation. The male approaches the female from behind, below, and slightly to the side followed by an attempt to introduce its gonopodium in her genital opening by bringing it forward. Sometimes there is no contact.	(Baerends, 1955; Houde, 1997; Liley, 1966)
Sigmoid display	Precede a true copulation with female cooperation. The male is placed in front or to the side of the female and bents its body laterally into an S or sigmoid shape. Sometimes it is accompanied by quivers, and he might remain in the same place or move over a short distance. There are several degrees of intensity.	(Baerends, 1955; Houde, 1997; Liley, 1966)

During the trials I registered: **(1)** Time to start sexual behaviour (latency), **(2)** Frequency of gonopodial swings, **(3)** Frequency of gonopodial thrusts (and towards which female the focal male directed this behaviour), **(4)** Frequency of sigmoid displays (and towards which female the focal male directed this behaviour), and **(5)** Duration of sigmoid displays. Preference for one female was assumed when the male was a maximum of one body length away from her.

From the behaviours above I obtained:

1. **Reproductive effort.** The sum of frequencies of sigmoid displays and gonopodial thrusts from a focal male towards both females.
2. **Mate choice.** The preference of a focal male to perform any mating tactic towards the big female or the small female. It is obtained by dividing the reproductive effort towards the big female by the reproductive effort. *If the result obtained equals 0.5, then the male´s preference is the same for both females; if it is < 0.5, then there is a preference for the small female; if it is > 0.5, then the big female is preferred by the male.
3. **Mating strategy.** The preference of a focal male to use the sigmoid display tactic or the gonopodial thrust tactic prior to a copulation attempt. It is obtained by dividing the frequency of sigmoid displays by the reproductive effort. *If the result obtained equals 0.5, then the male uses both tactics equally; if it is < 0.5, then there is a preference for using the thrusting tactic; if it is > 0.5, then the courtship display is preferred by the male.

Noise features and measurements

A well-defined acoustic stimulus (i.e., white noise) was used rather than artificial noise of single frequency (i.e., isolated tones) or playback recordings of anthropogenic activity (e.g., boat engine), as it represents more realistic and generic noise that a fish might encounter in shallow waters (Scholik & Yan, 2002; Short et al., 2020).

White noise was artificially generated with Audacity v. 3.2.5 (available from https://www.audacityteam.org/) in WAV-file format (32 bits, 44.1 kHz sampling rate), amplified to maximum level. White noise oscillates in a defined frequency range, being generally the audible sound spectrum for humans (20-20000 Hz). This range of frequencies include those expected to cover the hearing range of the Trinidadian guppy based on current understanding for related species as mentioned before (Schulz-Mirbach et al., 2010). We used 30 s ramps to fade in and fade out noise in order to get smooth transitions. 4 min of constant noise were played, followed by 1 min of silence. We used intermittent white noise (pulses) because continuous noise could lead to habituation (Bruintjes & Radford, 2013) and it has stronger effects than the last one (Shafiei et al., 2015). The noise file was played using VLC media (v. 3.5.4) player, through a speaker (JBL Clip 3; frequency response 120-20000 Hz) wrapped with plastic film to protect it from the water as it was not waterproof.

To confirm that males indeed perceived different noise levels between treatments, sound pressure and particle motion were both measured. For sound pressure measurement, we used an omnidirectional hydrophone (*Aquarian Audio & Scientific*, AS-1; sensitivity -208 dBV re 1 µPa, frequency response 1 Hz to 100 kHz) connected to a *Zoom H4n-PRO* recorder (sampling rate 44.1 kHz, 16 bit) (Figure 9). For particle motion measurement, an accelerometer (*Brüel & Kjær*, 4533-B-001; sensitivity 1 mV/ms^2, frequency response 0.2-12800 Hz) was used (Figure 9).

Figure 9. Hydrophone (left) and accelerometer (right).

Both devices were attached to a rigid support and then positioned inside each individual container (in the long-term effects experiment) and inside the observation tank (in the immediate effects experiment). In the case of the accelerometer, the sensitive region of the piezoelectric head was oriented toward the speaker. In both cases, the device was placed at a distance of 15 cm from the speaker (the distance of the individual tanks to the speaker, see Figure 6).

Several 10-second recordings with the hydrophone were made. Each recording was transferred to the sound analysis software Raven Pro v. 1.6.4 (K. Lisa Yang Center for Conservation Bioacoustics, 2023) to visualise its waveform, spectrogram, power spectral density, and the acoustic parameters selected (Table 3). Later, an average of all measurements was obtained (Table 4).

Table 3. Description of the selected measurements as defined in Raven Pro (Charif et al., 2010).

Measurement	Description	Units
RMS Amplitude	Root-mean-square amplitude of the selected part of the signal. The average amplitude is not used in the case of waveforms because it would result in cero, thus, RMS values are commonly used.	U
Max Amplitude	The maximum of all the sample values in the selection.	U

Table 4. Acoustic parameter variations between control and noise treatments in both experiments.

Acoustic parameters	Long-term effects		Immediate effects	
	Control	Noise	Control	Noise
RMS Amplitude (U)	1530	6947	277	8775
Max Amplitude (U)	8260	30271	1424	32766

Since our recordings were not calibrated, values of dimensionless sample units (U) were obtained. However, based on estimates made from the sound emission system, it was expected that the average sound pressure level was approximately 153.5 dB re 1 μPa in the noise treatments. To obtain this value we measured the dB-level of the noise recording in air, then we added to it 25.5 dB to get a comparable intensity level underwater, then another 36 dB are required due to the higher acoustic impedance of water compared to air (Slabbekoorn et al., 2010). Based on a previous literature review (Zavala-Rodríguez, unpublished data), most studies have employed acoustic stimuli with sound pressure levels around 152 dB re 1 μPa. In fact, according to the U.S. National Marine Fisheries Service, 150 dB re 1 μPa (RMS) is the sound pressure level that may result in onset of behavioural effects (Popper & Hawkins, 2019).

From the individual waveforms and spectrograms of each recording, it was evident that sound levels strongly differed between both experimental treatments (Figures 10 and 11). Waveforms show the relative intensity of the signal, which was much bigger when the white noise recording was played. Whereas the spectrograms represent at which points the relative power is greater (i.e., at which frequencies the noise intensity is highest), there being a wider range of frequencies covered when noise was present.

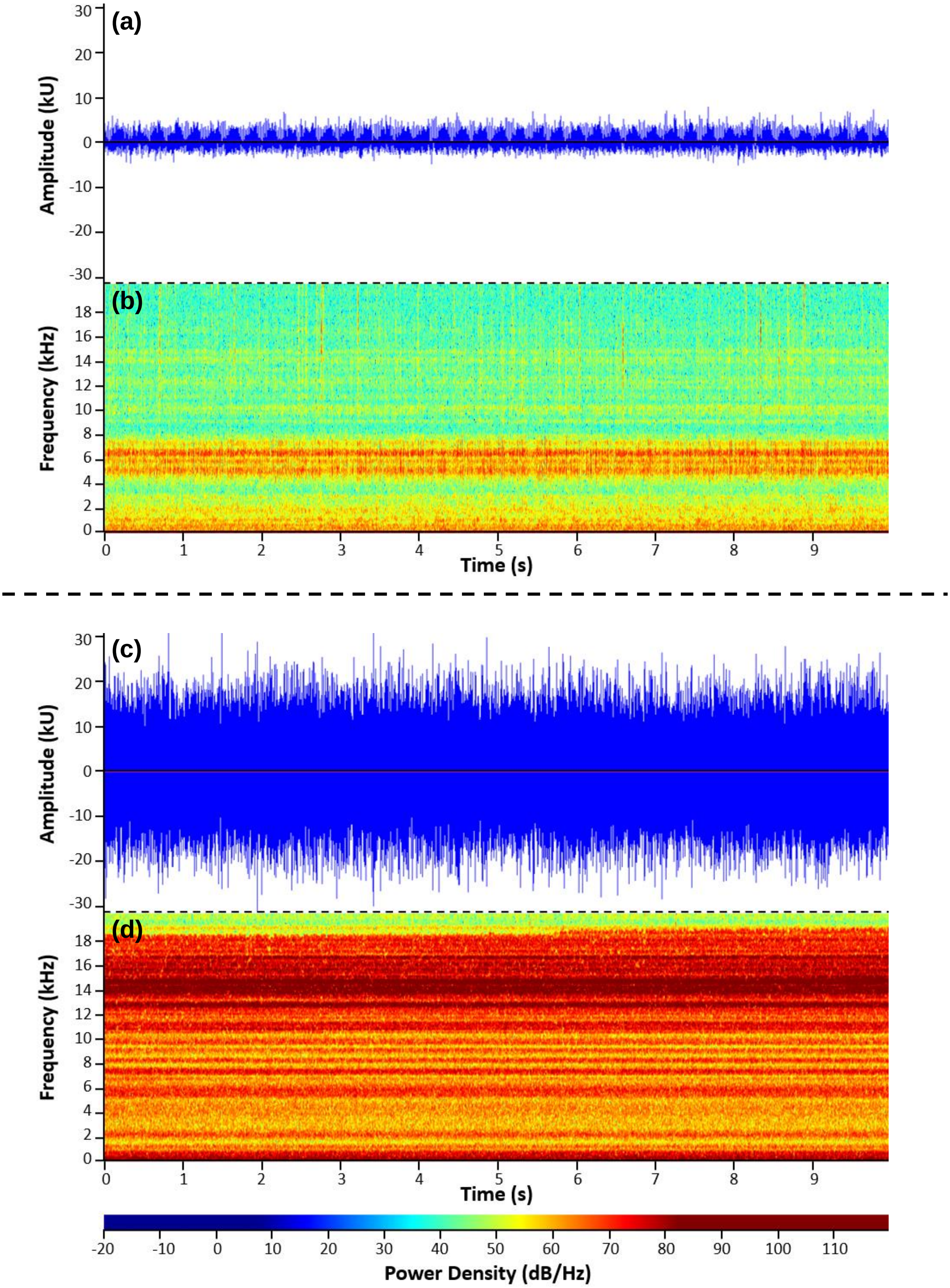

Figure 10. Acoustic characteristics in the long-term effects experiment. Waveform (a) and spectrogram in the range 0-20 000 Hz (b) of the control treatment. Waveform (c) and spectrogram in the range 0-20 000 Hz (d) of the noise treatment. Graphs were obtained from a single measurement.

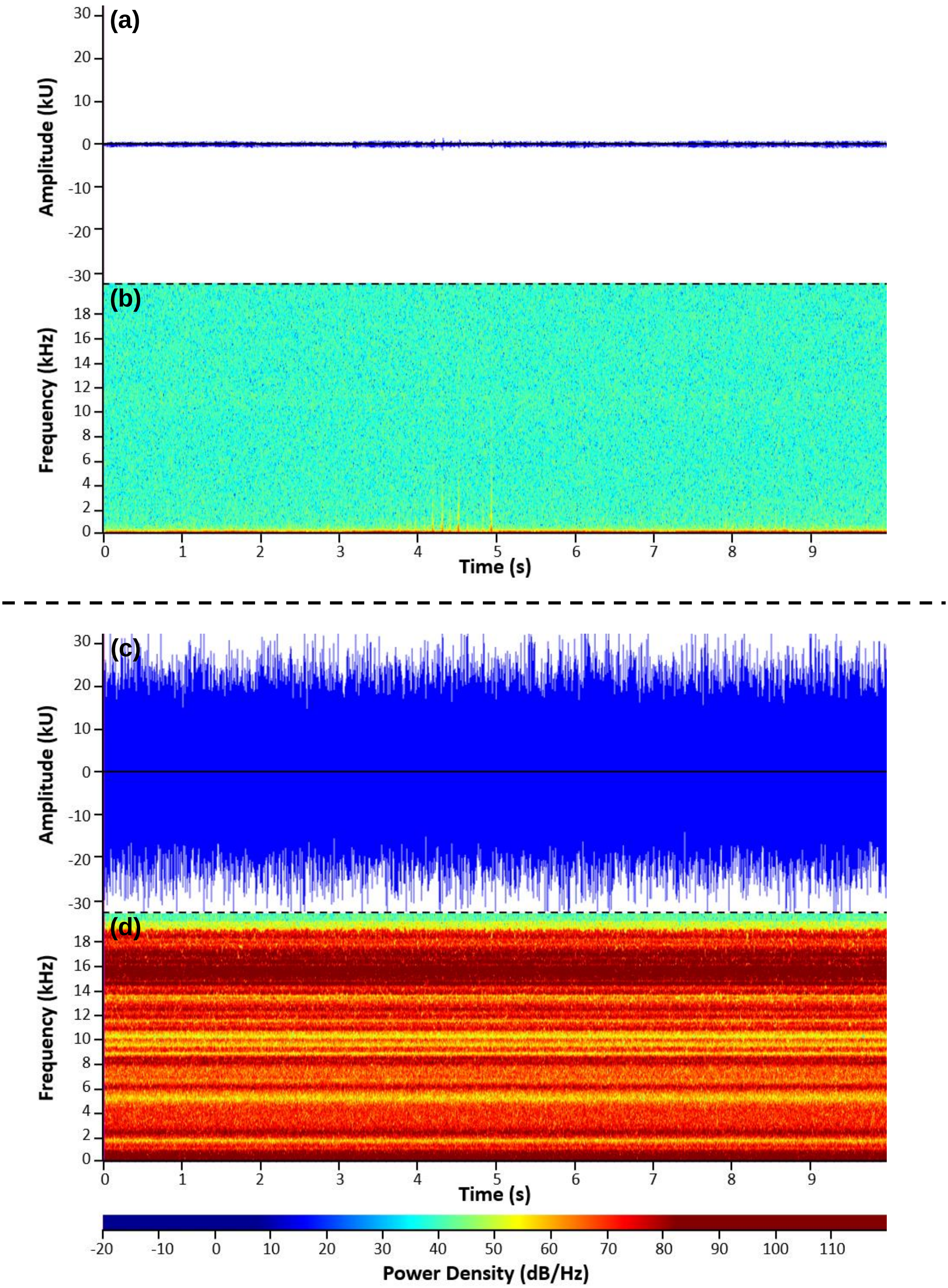

Figure 11. Acoustic characteristics in the immediate effects experiment. Waveform (a) and spectrogram in the range 0-20 000 Hz (b) of the control treatment. Waveform (c) and spectrogram in the range 0-20 000 Hz (d) of the noise treatment. Graphs were obtained from a single measurement.

The Power Spectral Density in Figures 12 and 13 show that the noise treatments had considerably higher sound levels (less negative readings indicate a louder signal) across almost the whole frequency range in comparison with the control treatments. Nonetheless, in the long-term effects experiments some frequencies had similar power density levels in both treatments, for example around 5-7 kHz (Figure 12). This overlap may be due to the low-frequency noise generated by the filter, which remained on during the whole experiment for the well-being of the fish.

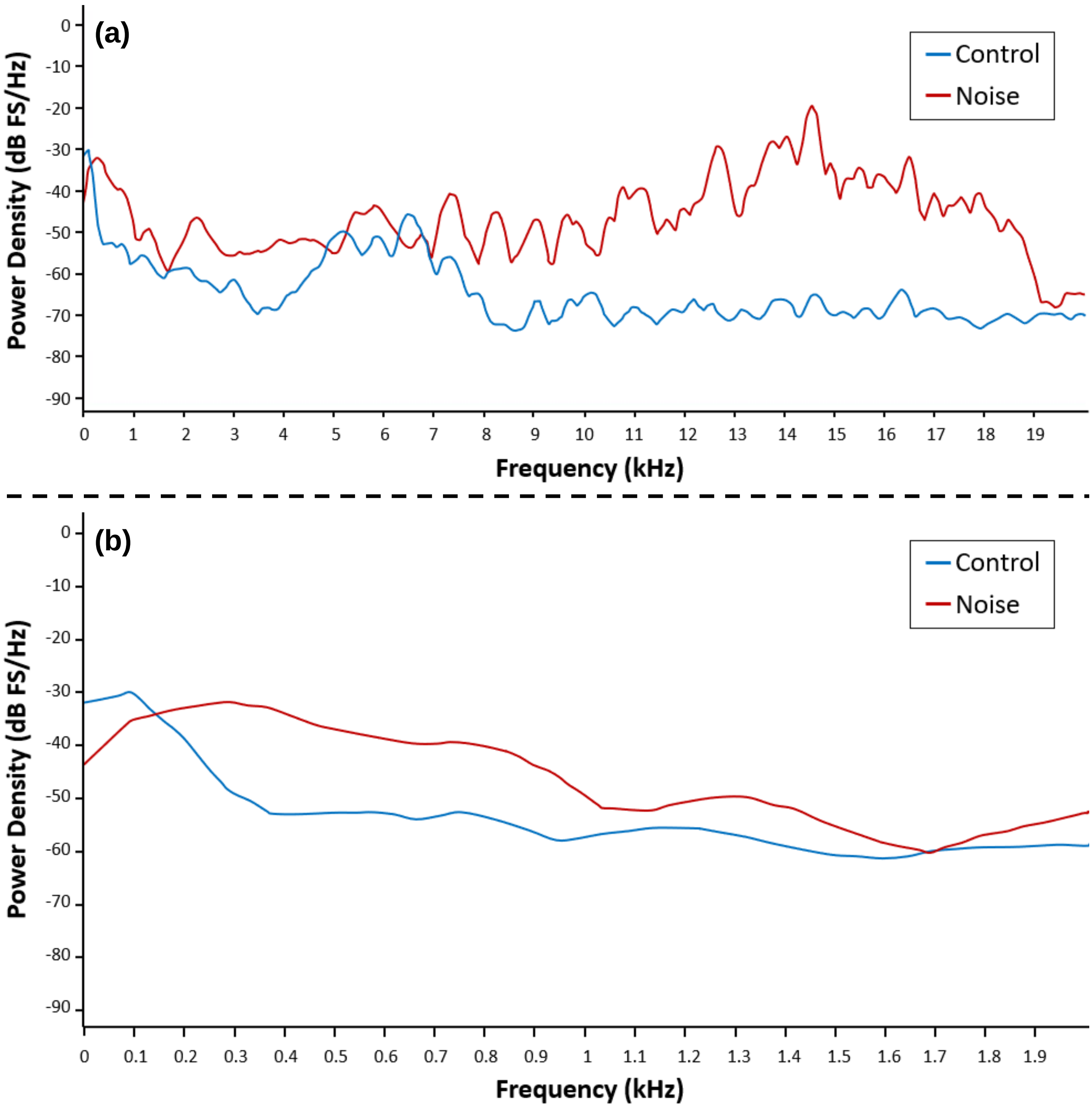

Figure 12. Sample of the Power Spectral Density of both treatments in the long-term effects experiments. (a) Graph obtained from a single measurement**.** Zoom in the 0-2 kHz range (b) is also shown.

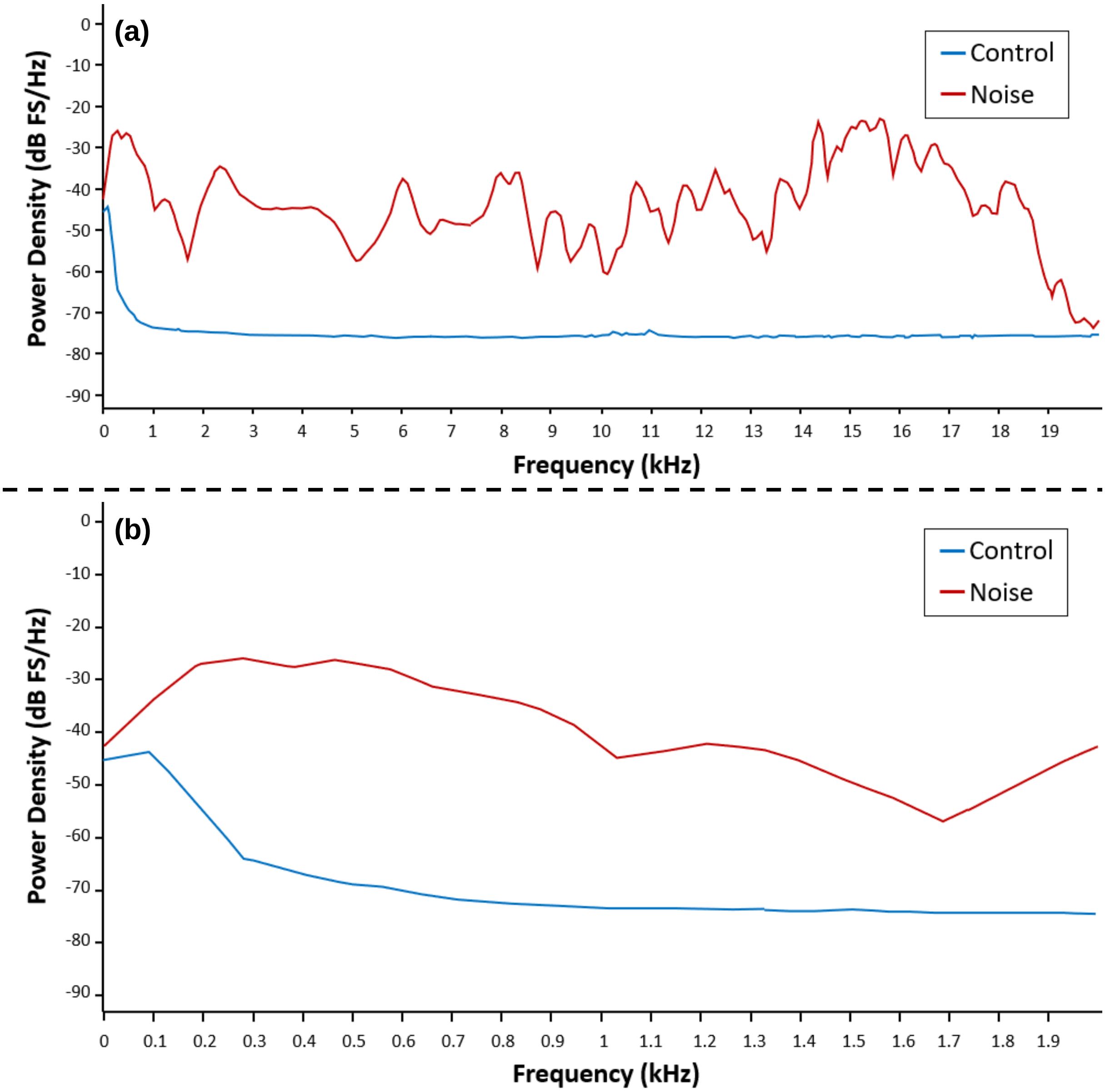

Figure 13. Sample of the Power Spectral Density of both treatments in the immediate effects experiments. (a) Graph obtained from a single measurement**.** Zoom in the 0-2 kHz range (b) is also shown.

Measurements with the accelerometer were visualised through an oscilloscope (*Tektronix* TBS 1102B-EDU) and data captures were saved in a usb. Subsequently, registered values in volts were analysed and converted to particle acceleration units. Graphs were obtained by averaging all measurements (Figure 14); from these, it can be clearly appreciated that particle acceleration levels in the noise treatment oscillated over a wider range of values compared to levels in control conditions.

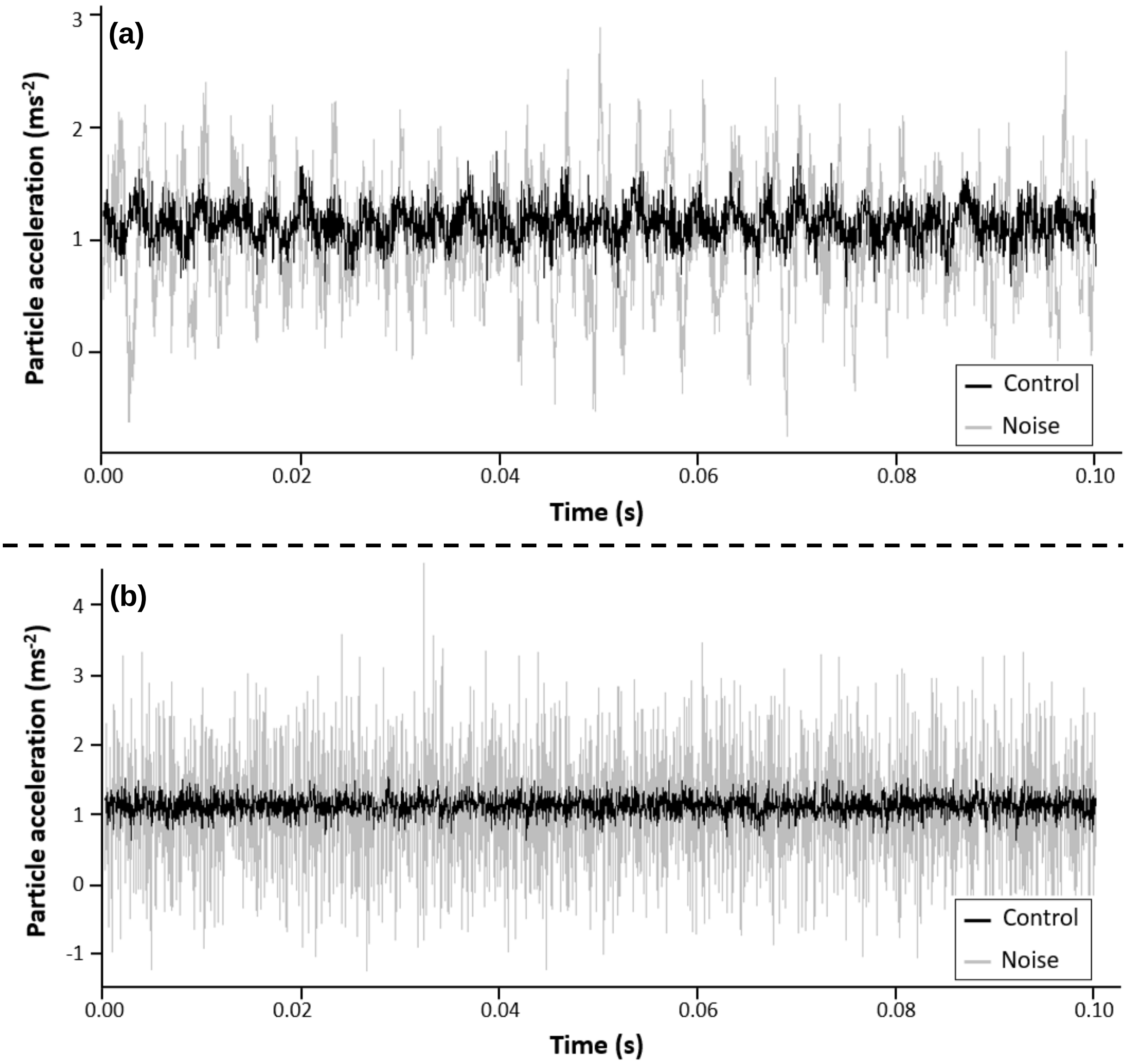

Figure 14. Average particle acceleration recorded in both treatments in the long-term (a) and immediate effects (b) experiment. Values captured in 100 milliseconds.

Morphological and colouration analyses

Before introduction to acclimatisation tanks, each male's body length was measured using a digital calliper (*Trupper*) from the snout to the caudal peduncle (excluding fins).

Morphological and colouration analyses were only performed in males from the long-term effects experiment. Before conducting Be trials, males were anaesthetised by dipping them in a clove oil solution (75 mg/L) so they could be weighed on an analytical balance and photographed on the left side under standardized conditions. A colouration analysis was performed using ImageJ v. 1.53e (Schneider et al., 2012) to obtain the percentage of body surface containing orange pigments. The latter was calculated as the proportion between the number of standard orange colour pixels and the number of pixels forming the total body area (including the caudal fin) (see Bertram et al., 2015).

Statistical analyses

All statistical analyses were performed using R v. 4.3.1 (R Core Team, 2023), with an alpha criterion of 0.05 established. Preliminary exploratory analyses were performed on all the tested behavioural traits, then normality of raw data was visually assessed from histograms and a Shapiro-Wilk test.

To evaluate the long-term effects, the difference between pre- and post-treatment trials (Af - Be) was obtained for each focal male's behaviour, and these difference scores were used in subsequent analyses. Males that did not exhibit any sexual behaviour during one or both trials (n = 15) were not considered for analyses of frequency of gonopodial thrusts, frequency and duration of sigmoid displays, reproductive effort, mate choice, and mating strategy; therefore, 17 males from the control treatment and 18 from the noise treatment were analysed. Generalised linear models (GLMs) with Gaussian distribution and identity link function were used to model the response variables of fish sexual behaviour (response variable) as a function of the treatment (explanatory variable).

To evaluate the immediate effects, the "glmmTMB library" (Brooks et al., 2017) was used to generate generalised linear mixed models (GLMMs) which modelled the response variables of fish sexual behaviour as a function of the treatment (explanatory variable, fixed factor). Fish identity was used as a random factor to prevent pseudoreplication. Males that did not exhibit any sexual behaviour during one or both trials (n = 4) were not considered for analyses of frequency of gonopodial thrusts, frequency and duration of sigmoid displays, reproductive effort, mate choice, and mating strategy; therefore, 22 males were analysed. GLMMs with Tweedie distribution and log link function (gonopodial swings), with Negative binomial and log link function (gonopodial thrusts, frequency and duration of sigmoid displays (zero-inflated), and reproductive effort), and with Beta and logit link function (mate choice and mating strategy) were used.

As experimental fish were placed in consecutive batches, batch number was treated as a fixed factor to rule out batch effect; but as it had no significant effect it was removed from the model (see Masud et al., 2020). Where appropriate, residuals were assessed for normality (Shapiro-Wilk test) and homogeneity of variance (Bartlett test). Estimated mean differences and their 95% confidence intervals (CI) were reported instead of *P* values since they offer a better understanding and characterisation of biological results (Nakagawa & Cuthill, 2007).

To determine the effect of the treatment on the probability that focal males started displaying sexual behaviours (latency), mixed-effects Cox regressions for a survival model were performed employing the "*survival*" and "*coxme*" libraries (Therneau, 2022, 2023). Type of treatment (and trial (be or Af) for long-term effects) were treated as explanatory variables, while male identity was treated as a random factor.

Weight, length, and orange pigment colouration were treated as covariates in separate GLMs because there is evidence that they can potentially affect male sexual behaviour (e.g., Nicoletto, 1993; Polverino et al., 2019). Previous to this, through Mann-Whitney-Wilcoxon tests, we assessed whether weight, length, and orange area differed between males from both treatments. Since mean differences and their CI are not appropriate when covariates are included, *P* values were reported here (Nakagawa & Cuthill, 2007). Because I did not find evidence that any of these parameters is correlated with the obtained results, I decided not to include these covariates in the second experiment (immediate effects).

RESULTS

Long-term effects of noise

Neither the trial number nor the treatment had an effect over the probability of focal males to start displaying sexual behaviours (Figure 15; Table 5).

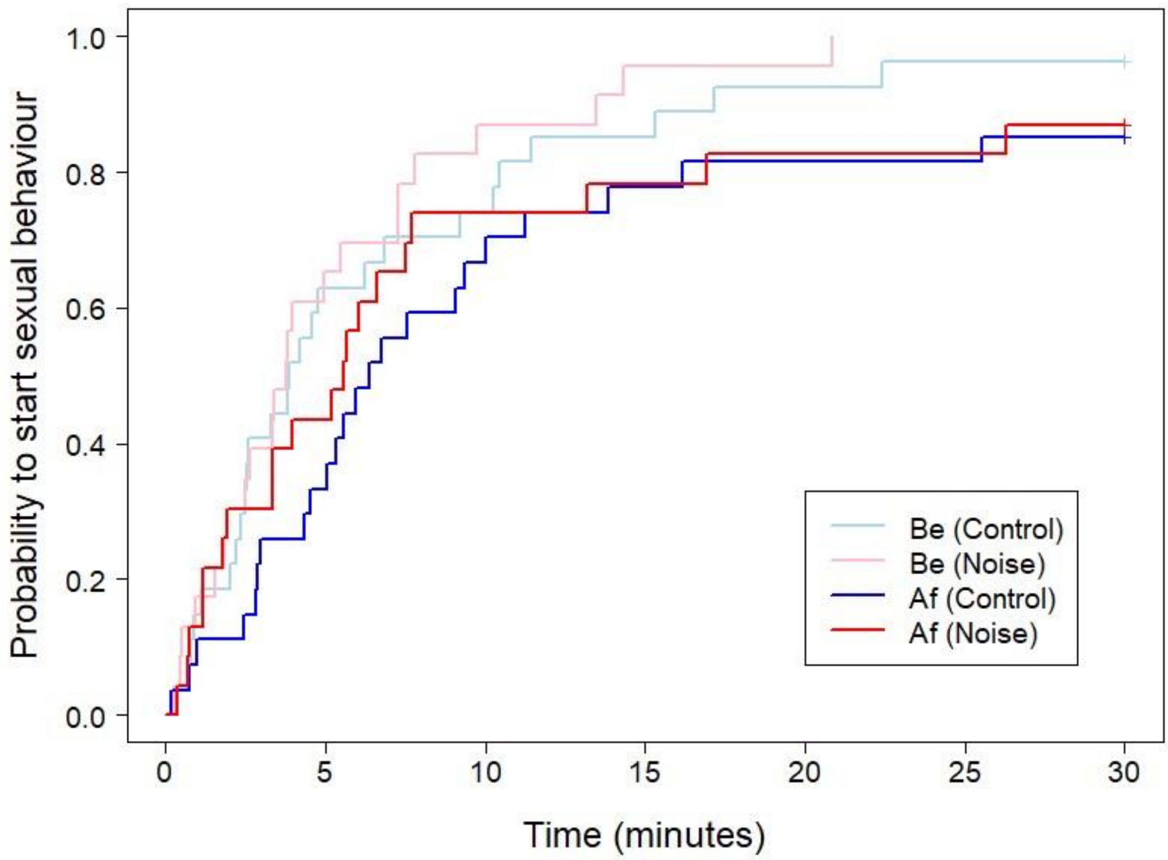

Figure 15. Probability of males to start displaying any sexual behaviour in four different situations.

Table 5. Mixed-effects Cox regression for a survival model.

Terms	Estimate	Standard error	z	*p*
Trial	-0.596	0.315	-1.89	0.059
Treatment	0.210	0.734	0.29	0.770
Trial*Treatment	0.069	0.453	0.15	0.880

The treatment was not an important predictor of the variation in the number of times focal males swung their gonopodium, nor the frequency of gonopodial thrusts they performed (Figure 16a, b; Table 6). However, variation in both the frequency and duration of sigmoid displays between treatments was observed in focal males (Figure 16c, d; Table 6).

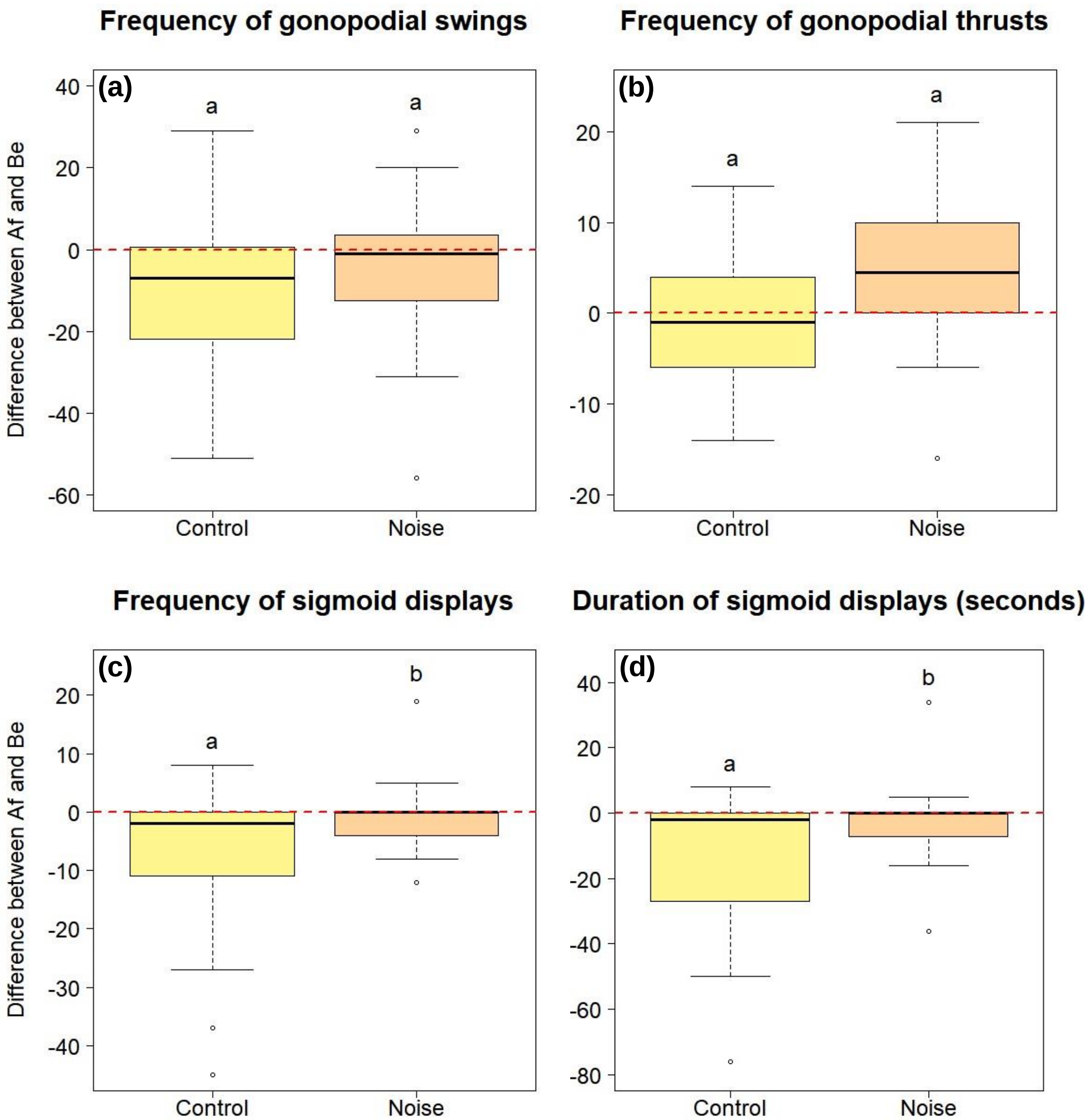

Figure 16. Median ± IQR difference (Af - Be) and the 5th and 95th percentiles in (a) the frequency of gonopodial swings (control n = 27, noise n = 23), (b) the frequency of gonopodial thrusts (control n = 17, noise n = 18), (c) the frequency of sigmoid displays (control n = 17, noise n = 18), and (d) the duration of sigmoid displays (control n = 17, noise n = 18) between focal males in both treatments. Different letters above boxplots indicate significant differences.

Males from the noise treatment performed more mating tactics after exposure, which suggests that the variation in the reproductive effort was explained by the treatment to which males were exposed (Figure 17a; Table 6). On the other hand, the statistical analysis indicated that treatment was not an important factor in explaining the variation in mate choice, nor mating strategy of focal males (Figure 17b, c; Table 6).

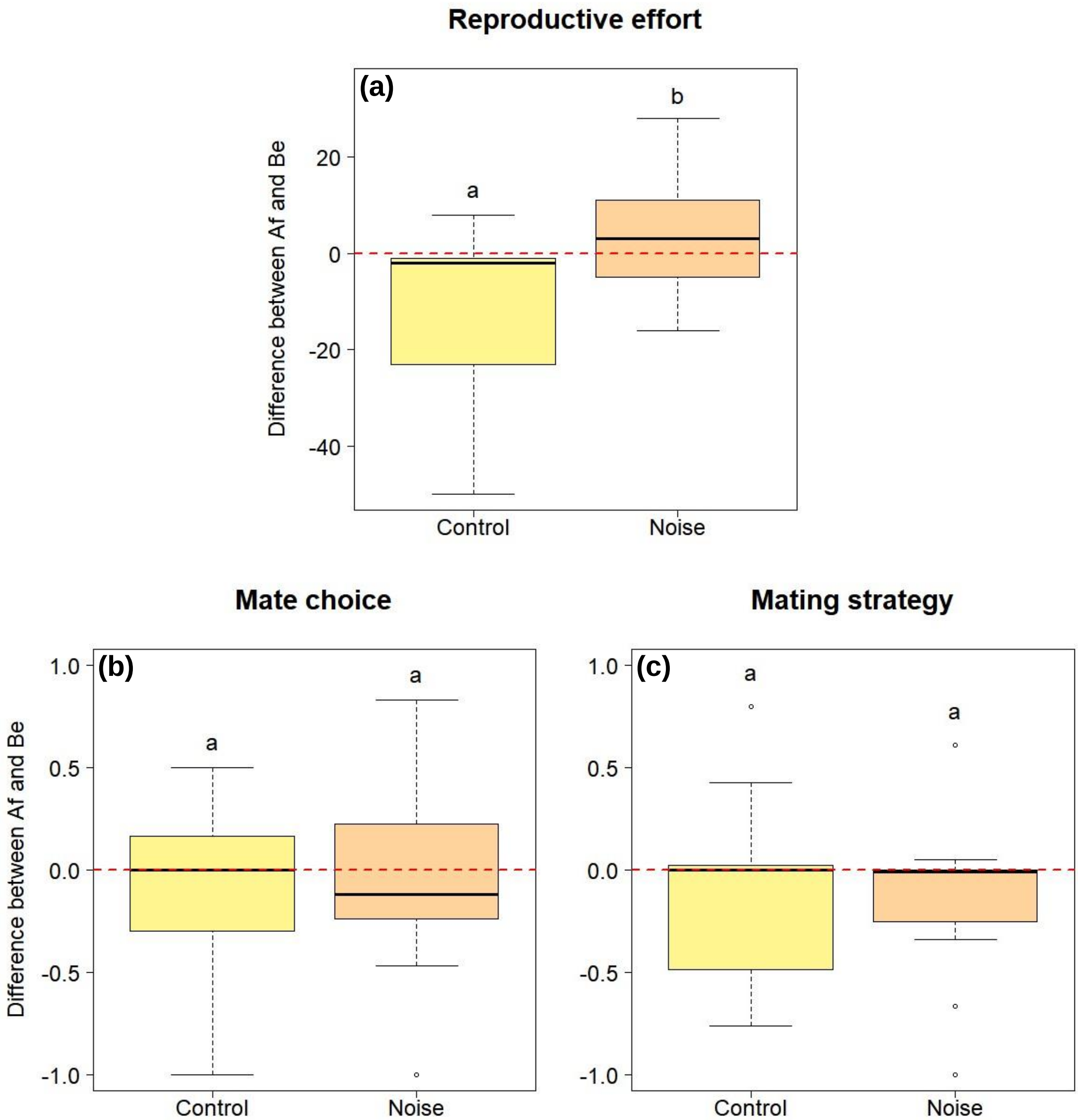

Figure 17. Median ± IQR difference (Af - Be) and the 5th and 95th percentiles in (a) the reproductive effort, (b) the mate choice, and (c) the mating strategy between focal males in both treatments (control n = 17, noise n = 18). Different letters above boxplots indicate significant differences. Values from 0 to 1 indicate that males increased their preference for the big female (b), and for the use of sigmoid displays (c) after the treatment. Values from 0 to -1 indicate that males decreased their preference for the big female (b), and for the use of sigmoid displays (c) after the treatment.

Table 6. Estimated mean differences (and their 95% confidence intervals, CI) between treatments for each response variable.

Response variable	Contrast	Estimate	95% CI Lower	95% CI Upper
Gonopodial swings	Noise - Control	5.657	-4.149	15.464
Gonopodial thrusts	Noise - Control	4.814	-0.557	10.184
Sigmoid displays (frequency)	Noise - Control	**7.699**	**0.098**	**15.301**
Sigmoid displays (duration)	Noise - Control	**13.516**	**0.392**	**26.641**
Reproductive effort	Noise - Control	**12.513**	**3.208**	**21.818**
Mate choice	Noise - Control	0.052	-0.230	0.334
Mating strategy	Noise - Control	0.011	-0.239	0.262

**Note*: Significant terms in bold (i.e., the estimated mean differences and their 95% CI do not overlap zero).

Males' standard length (Mann-Whitney-Wilcoxon test: W = 333.5, *P* = 0.661), weight (Mann-Whitney-Wilcoxon test: W = 339, *P* = 0.589), and orange pigmentation area (Mann-Whitney-Wilcoxon test: W = 355.5, *P* = 0.386) did not differ significantly between both treatment groups. None of these traits had an influence on any of the evaluated sexual behaviours of focal males (see Supplementary material, Table 9).

Immediate effects of noise

The treatment did not have an effect over the probability of focal males to start displaying sexual behaviours (Figure 18; Table 7).

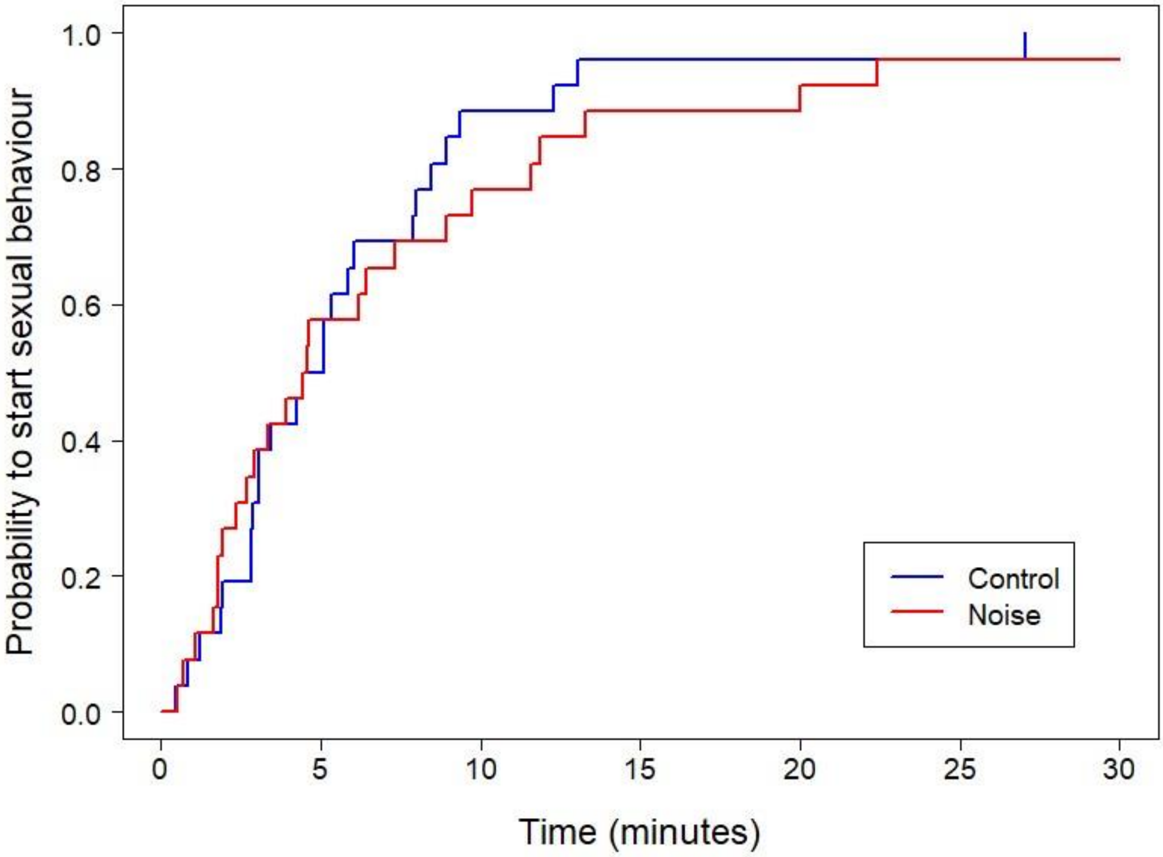

Figure 18. Probability of males to start displaying any sexual behaviour in two different situations.

Table 7. Mixed-effects Cox regression for a survival model.

Terms	Estimate	Standard error	z	*p*
Treatment	-0.178	0.291	-0.61	0.54

The treatment was not an important predictor of the variation in the number of times focal males swung their gonopodium, the frequency of gonopodial thrusts, nor the frequency and duration of sigmoid displays during exposure to noise (Figure 19; Table 8).

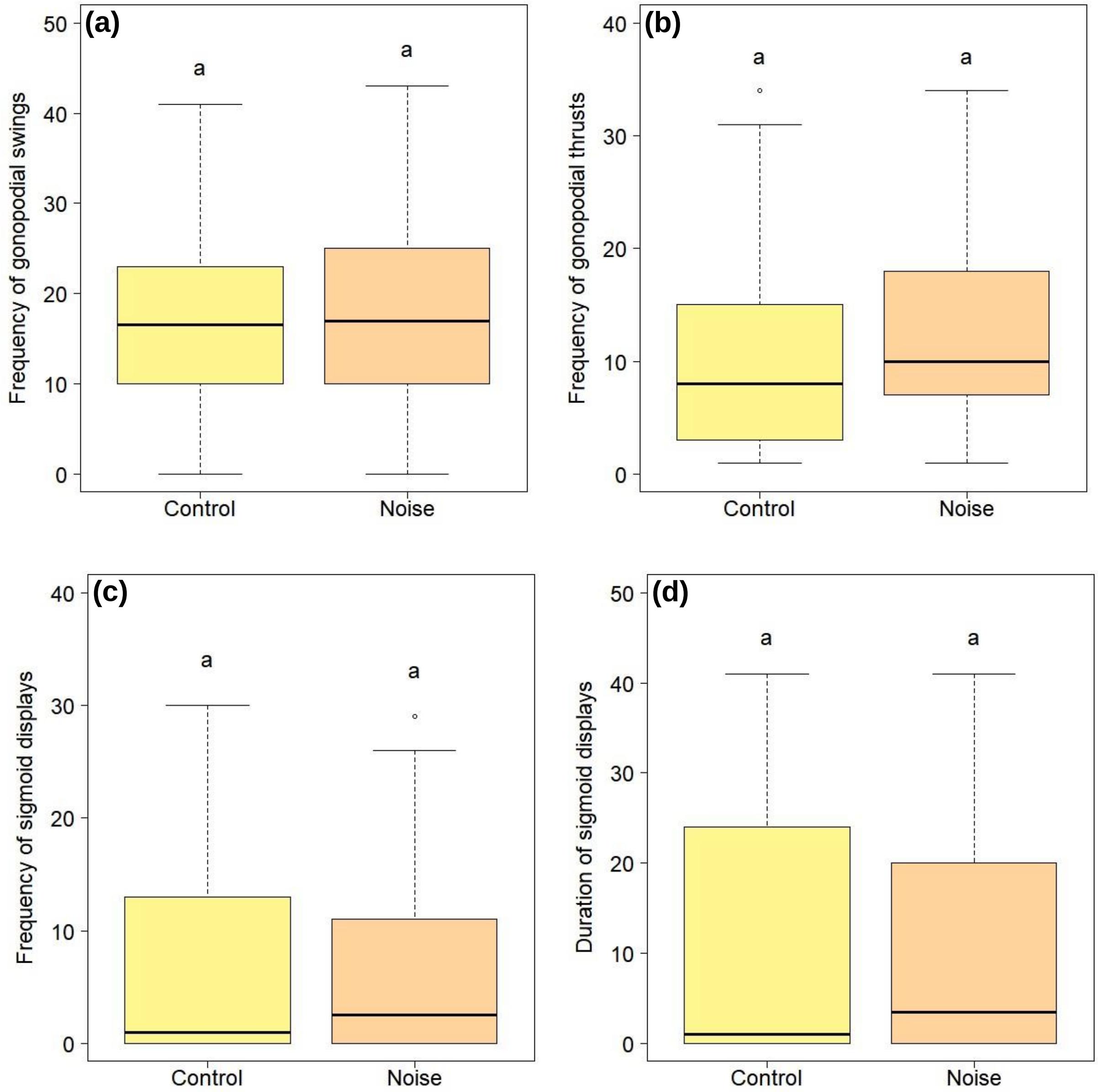

Figure 19. Median ± IQR, and the 5th and 95th percentiles in (a) the frequency of gonopodial swings (n = 26), (b) the frequency of gonopodial thrusts (n = 22), (c) the frequency of sigmoid displays (n = 22), and (d) the duration of sigmoid displays (n = 22) of focal males in both treatments.

The statistical analysis indicated that treatment was not an important factor in explaining the variation in the reproductive effort, mate choice, nor mating strategy (Figure 20; Table 8).

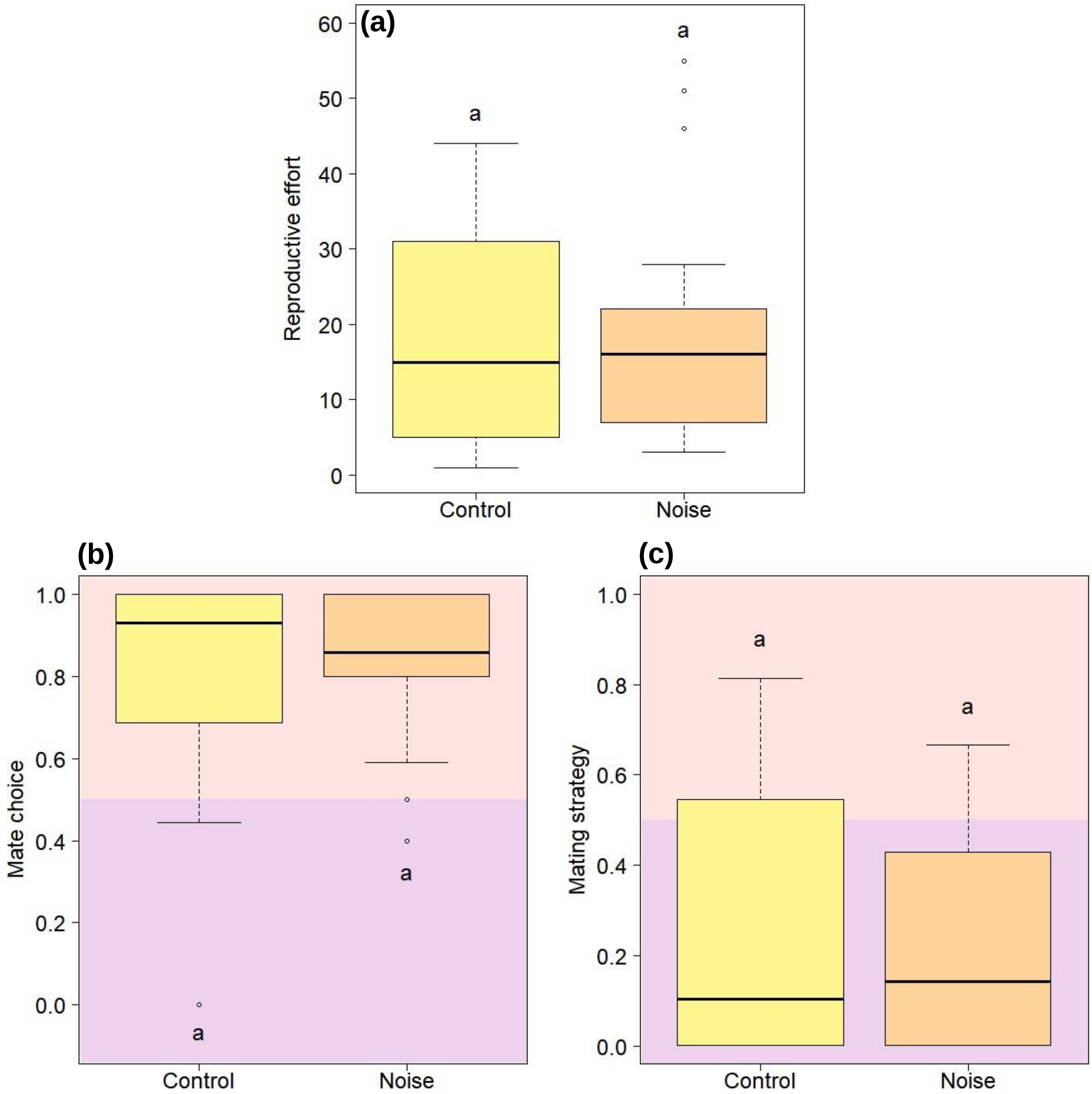

Figure 20. Median ± IQR, and the 5th and 95th percentiles in (a) the reproductive effort, (b) the mate choice, and (c) the mating strategy of focal males in both treatments (n = 22). Different letters above boxplots indicate significant differences. Values from 0 to 0.5 (purple colour in the scheme) indicate that the focal male preferred the small female (b), and to perform gonopodial thrusts (c). Values from 0.5 to 1 (pink colour in the scheme) indicate that the focal male preferred the big female (b), and to perform sigmoid displays (c).

Table 8. Estimated mean differences (and their 95% confidence intervals, CI) between treatments for each response variable.

Response variable	Contrast	Estimate	95% CI Lower	95% CI Upper
Gonopodial swings	Noise - Control	0.089	-0.263	0.442
Gonopodial thrusts	Noise - Control	0.155	-0.304	0.614
Sigmoid displays (frequency)	Noise - Control	-0.157	-0.974	0.660
Sigmoid displays (duration)	Noise - Control	-0.243	-1.123	0.636
Reproductive effort	Noise - Control	0.057	-0.422	0.537
Mate choice	Noise - Control	-0.002	-0.676	0.673
Mating strategy	Noise - Control	-0.060	-0.741	0.621

DISCUSSION

Disturbances caused by acoustic pollution differ from those of other stressors in that once the noise source is silenced or removed, no noise traces remain (Bruintjes et al., 2016). Therefore, we can expect that organisms either respond to noise only during real-time exposure (e.g., Bent et al., 2021a; Holt & Johnston, 2014; Rising et al., 2022) or that long-term effects remain (e.g., Amorim et al., 2022; de Jong et al., 2018a). Ideally, both responses must be assessed to fully depict the effects of noise.

In this study, I assessed the long-term and immediate effects of noise on the sexual behaviour of male guppies by comparing individuals exposed to a white noise and a control treatment. To test my hypothesis, I evaluated male´s behaviours before and after a four-day exposure, and during real-time exposure to the treatments. Contrary to my predictions, there were no immediate effects on behaviour; nevertheless, I found an increase in the reproductive effort of males after 4 days of noise exposure.

Effects on sexual behaviours and reproductive effort

Differences in the time it takes an animal to begin showing certain behaviours (i.e., latency) have been observed between individuals exposed or reared under particular environmental or social circumstances (e.g., Barbosa et al., 2013). In this study, it was predicted that male guppies would increase the latency to start showing sexual behaviours after or during exposure to noise, suggesting a decrease in their willingness to display these behaviours. However, I found that males' latency did not differ between treatments. Consistent with my findings but in a different taxon, Mediterranean field cricket males' latency to signal courtship displays was not influenced by man-made noise (Bent et al. 2021a).

In guppies, variation in sexual activity and effort devoted to mate usually depends on the energetic status or condition of males (Houde, 1997). A depressing effect on these traits can be caused by environmental stressors, such as high parasite loads (Houde & Torio, 1992; Kennedy et al., 1987), immune challenge (Encel et al., 2023), and food limitation (Devigili et al., 2013; Rahman et al., 2013, 2014). Since elements introduced to the environment through human activity can also exert this effect (e.g., Baatrup & Junge, 2001; Colgan et al., 1982; Rahman et al., 2022; Schröder & Peters, 1988), less time spent showing sexual interest in females under noisy conditions was expected.

Interestingly, and contrary to my predictions, males in the long-term effects experiment showed a greater reproductive effort in comparison with control males. It is not easy to interpret these results given the opposite findings in the literature. For instance, painted gobies decreased their acoustic and visual courtship after exposure to low-frequency noise (de Jong et al., 2018b); and green shore crabs (*Carcinus maenas*) diminished their mating behaviour in response to ship noise (Rising et al., 2022). Certainly, a positive effect of anthropogenic noise on behaviour (represented as an increase) has also been observed (Cox et al., 2018); for example, in nest care in *Chromis chromis* (Picciulin et al., 2010), erratic swimming in the black bullhead (*Ameiurus melas*) (Pieniazek et al., 2020), or hiding behaviour in a coral reef fish (*Dascyllus trimaculatus*) (Nedelec et al., 2016b). Nevertheless, it is worth noting that these behaviours usually appear to be responses to perceived danger. To our knowledge, this study is the first one that has found an increase in some behavioural traits that allow organisms to reproduce.

Care should be taken when drawing conclusions from these results. Even though male mating success is positively correlated with a higher reproductive effort in guppies, we cannot conclude that the observed increase will lead to a higher reproductive success.

Firstly, because we did not measure reproductive success *per se*, and secondly because males still performed more forced copulation attempts rather than courtships (see below), the first tactic being less expected to precede an insemination of the female (Kennedy et al., 1987).

Effects on mate choice and mating strategy

A change in males' preference for a particular female and one mating tactic was expected when exposed to noise. These preferences, as well as sexual behaviours, can be influenced by natural external forces (e.g., Endler, 1987; Guevara-Fiore & Endler, 2018; Head et al., 2010; Makowicz et al., 2010; Polverino et al., 2019), or by anthropogenic factors (e.g., Bertram et al., 2020). However, males in this study did not alter their mate choice nor their mating strategy. In fact, they almost always preferred to perform sexual behaviours towards the bigger female (Figure 20b; see Supplementary material, Figure 21), and to engage in more forced copulation attempts (Figure 20c; see Supplementary material, Figure 22), regardless of which treatment they underwent.

Concerning mate choice, our results suggest that noise does not act as a disruptive stimulus capable of altering the generalised preference of males to associate with larger females, a tendency which is possibly explained by the widely recognised positive association between body size and fecundity, and thus with reproductive success (Dosen & Montgomerie, 2004). The opposite has been observed in other organisms such as female Mediterranean field crickets, which often prefer to mate with males with higher-quality songs, but under traffic or white noise conditions their mate choice patterns alter (Bent et al., 2021b). Nevertheless, it is important to consider that in this last case only one signalling modality (acoustic) is intervening, while in our case there are two modalities (acoustic and visual).

Respecting mating strategy, it is worth noting that even though males exposed to noise for four days performed more sigmoid displays in comparison with the control group, gonopodial thrusting was still the most used tactic. A possible cause of this fixed preference might be given by the origin of the fish. Conspicuous courtship behaviours in wild guppies are adaptations to clear water conditions, and gonopodial thrusting to turbid waters (Luyten & Liley, 1985); since the lake where the population of guppies was collected had these last conditions (personal observation), males might rely more on thrusting. Furthermore, male sexual behaviour is influenced by the behaviour and reproductive status of females (Guevara-Fiore et al, 2010; Guevara-Fiore & Endler, 2018); for example, when females are unresponsive and displays are not expected to be effective, gonopodial thrusting is preferred (Houde, 1997). In this project, only non-virgin females were used, and this could also explain the lack of change in mating strategy.

Unexpected outcomes

Not finding immediate effects in this study was surprising. A possible, but unlikely, explanation to this could be given by the fact that while males in the long-term effects experiment received the acoustic stimuli from their individual tanks, males in the immediate effects experiment could swim closer and be more directly exposed to the noise source. After exposure to very high noise levels, some animals exhibit an increase in their auditory threshold (i.e., a decline in auditory sensitivity) (e.g., Scholik & Yan, 2001; Smith et al., 2004), or a more intense response in the form of hearing loss, mainly due to damage of the auditory tissues (i.e., sensory hair cells of the inner ear) (e.g., Mickle et al., 2019). Both cases lead to a reduction in the perception of noise and eventually a reduction in stress (Smith et al., 2004). Nevertheless, it is more likely that there were no immediate effects at all, or that more days of exposure might be required to elicit any response.

Another unexpected outcome was observed in the courtships in the long-term experiment: after the noise, treatment both the number and duration of sigmoid displays remained quite constant; however, in the control group males suffered a slight significant decrease. Some parameters about sperm quality were also similarly altered (López-Flores, 2024). Fish within both treatments were treated identically throughout the experiment, and had the same temperature, type and amount of food, water quality levels, light conditions, and minimal handling stress. Therefore, it is unlikely that experimental conditions are responsible for the decrease of these behaviours after the treatment period. Instead, other possible reasons can be stated.

For example, the female visual and olfactory stimuli received by males for sperm production may have been small either because of dim lighting within the opaque tanks (and therefore poor visibility), a low female density, or biassed visits from females to focal males (because some males' phenotypes in guppies are more attractive than others) (Houde, 1997). Even though we took into consideration the number of days needed for the males to recover after being stripped, it might be possible that four days were not enough to fully replenish their spermatophores reserves. Since there is a positive correlation between sperm reserves and sexual activity in guppies (Bozynski & Liley, 2003; Guevara-Fiore, unpublished data), the observed decrease in sexual behaviours might be explained by this.

Isolation of males in individual containers was necessary in this experiment to guarantee that all focal individuals received the same noise stimulus. However, this element of our experimental design could have generated an additional and undesired stress for the fish, which could be an alternative explanation for our results. This has been observed in male rainbow trout (*Oncorhynchus mykiss*), which increased their cortisol levels almost fourfold when confined alone (Bobe & Labbé, 2010). Higher stress levels in our focal males due to isolation could have resulted in a diminished sexual activity.

Regardless of the causes of this decline in courtships in the control group, it would be interesting to investigate why courtships remained unaltered in males after being exposed to noise.

Further insights and recommendations

Interspecific differences have been observed in the responses of organisms to anthropogenic noise (Fleissner et al., 2022; Pieniazek et al., 2020; Voellmy et al., 2014; but see Wysocki et al., 2006). Also, the same noise source might not equally affect all fish (see for example Scholik & Yan, 2001, 2002); therefore, the outcomes of this study must not be extrapolated to other taxa. In addition, behavioural responses to stimuli can also greatly vary between individuals (Francis & Barber, 2013) and populations (Devigili et al., 2013). Here we focused purely on males' behaviour, but since it is unlikely that only one sex is affected and the other one remains intact under the same conditions, especially if they influence each other's behaviour (Head et al., 2010), it would be very interesting to evaluate how noise affected females in these experiments.

An obvious disturbance caused by the presence of noise was never observed in the noise treatments; instead, the focal male and females swam calmly near the speaker during the trials. However, even if there are no evident physical changes in response to noise exposure, animals can show immediate physiological changes known as 'stress responses', which may include hormonal, immune or autonomic responses (Popper & Hawkins, 2019). One way of getting a better picture of the immediate impacts of noise could be reached by evaluating physiological responses, since even though detrimental effects caused by stressors are expected, behavioural changes may also be neutral or beneficial by alleviating the noise impact on physiological effects (Slabbekoorn, 2012).

Also, the fact that we do not know a lot about detection of acoustic signals in guppies, how much they rely on pressure and on particle motion for hearing, and the contribution of the lateral line system in detection of vibration, are important points to consider when interpreting the results of this project.

Noise can be particularly striking in organisms that communicate acoustically, as this makes them more prone to suffer from noise masking (Popper & Hawkins, 2019; Radford et al., 2014), and thus the potential acoustic signal space is decreased (Potvin, 2017). But although many animals are soniferous (i.e., that produce sound), others are not. Despite the growing interest in studying the effects of noise on animals, few studies have focused on those species that lack acoustic communication. However, those who have explored this have uncovered that non-soniferous animals respond to man-made noise, specially by exhibiting a suite of visual responses (e.g., de Jong et al., 2018a; Gordon & Uetz, 2012; Holmes et al., 2017; Rising et al., 2022). The findings in this study reinforce this body of literature. Besides, they also support the belief that noise generated by common holding conditions inside aquaria can interfere in organisms' behaviour and physiology (e.g., Gutscher et al., 2011; Wysocki et al., 2007).

Finally, the shifts that emerge from exposure to noise could potentially incur a cost (e.g., compromise the attractiveness of signals for potential mates) or become maladaptive (e.g., lead to inefficient decisions) (Francis & Barber, 2013); therefore, consistent detrimental health effects in the long term might be expected. A few studies have sought to determine the influence of noise on reproductive success directly, and while some of them have found detrimental effects (e.g., Amorim et al., 2022), some others have not observed any disruption (e.g., Bruintjes & Radford, 2014). In our case, it is suggested that males inhabiting noisy environments might have a higher reproductive success than those living in sites not reached by noise. Thus, it is important not only to evaluate effects at individual level, but also on current and future reproductive success and at population, community, and ecosystem levels (Jones & Reynolds, 1997). Conducting studies that seek to elucidate ultimate fitness consequences by directly assessing reproductive success or survival might not only result interesting but are also necessary to assess if selection of the most efficient signals occurs in the long-term (i.e., adaptation), and in this way achieve a broader perspective of the effects of man-made activity on the natural evolution of species.

CONCLUSION

Taken together, these results indicate that the sexual behaviour of males of a freshwater non-soniferous fish species is influenced by the long-term, but not the immediate, effects of acoustic pollution under laboratory conditions. Against expectations, reproductive effort increased after a four-day exposure to noise. These findings suggest that apparently male guppies are not negatively affected by noise; however, they represent evidence of the potential disturbances that noise can introduce in aquatic environments, and that it is able to affect even those organisms that do not communicate acoustically.

Our results should be interpreted with some caution, and extrapolation to other species must be avoided. Certainly, improvements in the experimental design of this study could contribute to having a better and closer representation of noise in nature, and therefore clearer outcomes. Finally, future research efforts on the characterisation of the effects of this pervasive pollutant on ecosystems should consider concrete fitness consequences to get a full picture of the extent of these impacts.

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APPENDIX 1. Glossary

Amplitude: Maximum distance that a vibrating particle moves from its equilibrium value. A sound wave's amplitude relates to the change in pressure; if it increases the sound is perceived as louder, while if it decreases then it is perceived as softer.

Attenuation: In acoustics, it refers to the reduction of a signal and its propagation. It may be caused by absorption, divergence, or scattering.

Audiogram: Also known as hearing threshold curve; it is the graphic representation of the hearing sensitivity of an animal. It shows the sound level that an animal requires to detect sounds across the frequencies that they can hear.

Auditory evoked potential (AEP): Summed potentials that are evoked by acoustic stimuli. It is a valuable element when measuring hearing sensitivity through electrophysiological responses of the auditory system. The technique to record this electrical activity employs electrodes, the reference and the measuring ones, positioned on the surface of the head while sounds with different intensities and frequencies are being played.

Auditory sensitivity threshold: Softest sound (i.e., at a minimum intensity) that an animal can reliably perceive at a specific frequency. In an audiogram it represents the lower limit of what the animals can hear.

Frequency: Number of cycles of a wave per second, expressed in Hertz (Hz). If the frequency of a sound increases, we get a higher pitched sound, while if it decreases then we get a low-pitched sound.

Gaussian white noise: Noise that contains many frequencies, all of them presented with equal intensities. Usually, the frequency range covered by white noise is 20-20000 Hz, which represents the audible sound spectrum for humans.

Intensity: Average amount of sound power (i.e., acoustic energy) radiated away from a source per unit area in a specified direction. Relative sound intensities are often expressed in decibels (dB).

Lombard effect: Response that occurs as a compensation for elevated noise levels, which consists of the increase in the amplitude of signals emitted by an animal. This effect has been mainly seen in birds, but recently in some fishes too.

Masking: Interference in the detection, localisation, and recognition of biologically relevant sounds by other sounds or noise. Masking of the acoustic signals in aquatic environments is mainly caused by underwater traffic, but also by traffic noise.

Oscilloscope: Instrument that graphically shows electrical signals and its change over time. It is connected to a device that creates an electrical signal in response to physical stimuli, in our case an accelerometer that responds to sound.

Pile driving: Process which employs a mechanical device to drive piles into the soil, which is widely used for the construction of bridges, harbours, wind farms, and other offshore structures. This activity produces elevated sound pressure levels in both terrestrial and underwater environments.

Power spectral density (PSD): Also known as energy spectral density (ESD); indicates the power or energy in a specified frequency band divided by its bandwidth.

Priming: In this context, it refers to the physiological changes released by the stimuli provided by females. These include the production of sexual components such as sperm and seminal fluid, and sexual behaviours.

Rarefaction: The instantaneous and local reduction in density of the medium resulting from the passage of a sound wave.

Reverberation: Persistence of sound waves after its generation has stopped.

Seismic exploration: Method of seafloor mapping that works by generating propagating waves which are reflected or refracted by different layers in the seafloor.

Soniferous species: Species that communicate through acoustic signals.

Sound level: It is the logarithm of a power ratio. It is usually expressed in units of decibel (dB) with reference values.

Spectrogram: Three-dimensional plot that shows how the spectrum of a sound varies over time. Frequency is represented on the vertical axis, time in the horizontal one, and the relative power at a given point in time and frequency with different colours.

Waveform: Graphic representation of the change in pressure of a sound wave through time. The relative pressure is related to the intensity of the sound.

Weberian ossicles: Morphological adaptation present in Ostariophysan fishes, which consists of a series of movable bones that connect the swim bladder to the inner ear. The expansion or contraction of the swim bladder results in the motion of these ossicles, and thus in the amplification of the acoustic waves. This structure endows fish with finer hearing capacities.

APPENDIX 2. Supplementary material

Table 9. GLMs with morphometric traits and colouration of focal males as predictor factors.

Response variables	Terms	d.f.	Deviance	Pr (> Chi)
Gonopodial swings	Length	1	22.02	0.793
	Weight	1	25.67	0.777
	Orange	1	610.49	0.166
Gonopodial thrusts	Length	1	0.670	0.925
	Weight	1	1.790	0.878
	Orange	1	3.270	0.836
Sigmoid display (frequency)	Length	1	268.060	0.170
	Weight	1	165.091	0.282
	Orange	1	5.198	0.849
Sigmoid display (duration)	Length	1	502.38	0.279
	Weight	1	517.21	0.272
	Orange	1	220.09	0.474
Reproductive effort	Length	1	241.931	0.314
	Weight	1	201.265	0.359
	Orange	1	16.713	0.791
Mate choice	Length	1	0.011	0.809
	Weight	1	0.122	0.419
	Orange	1	0.064	0.558
Mating strategy	Length	1	0.322	0.128
	Weight	1	0.041	0.587
	Orange	1	0.044	0.575

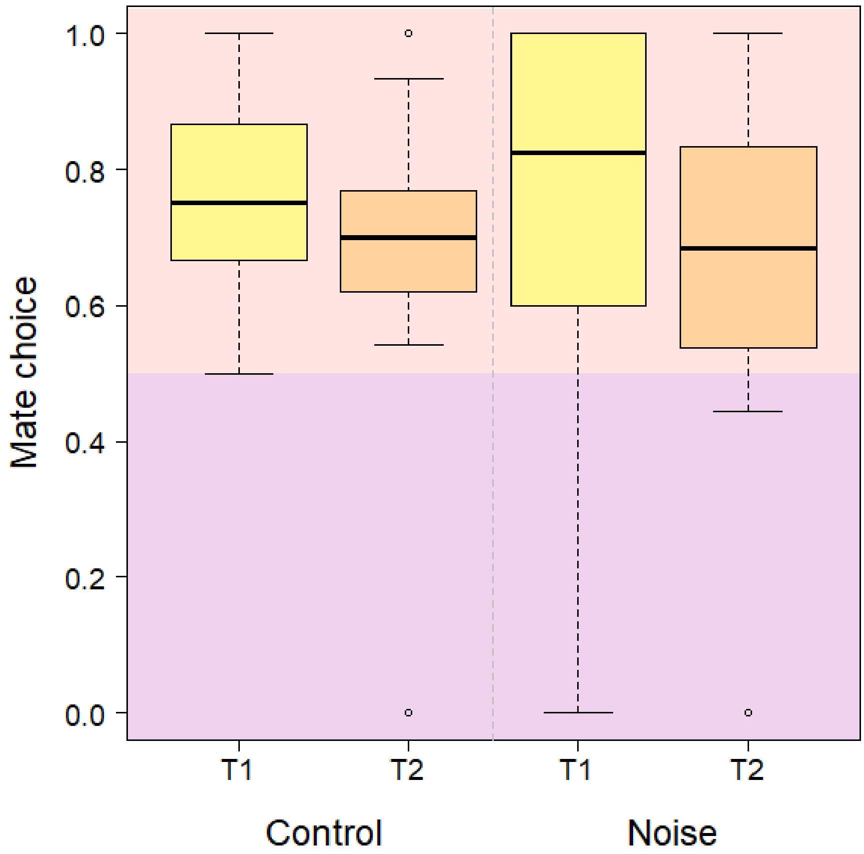


Figure 21. Median ± IQR of the mate choice of focal males before and after both treatments (control n = 17, noise n = 18), and the 5th and 95th percentiles. Values from 0 to 0.5 (purple colour in the scheme) indicate that the focal male preferred the small female. Values from 0.5 to 1 (pink colour in the scheme) indicate that the focal male preferred the big female.

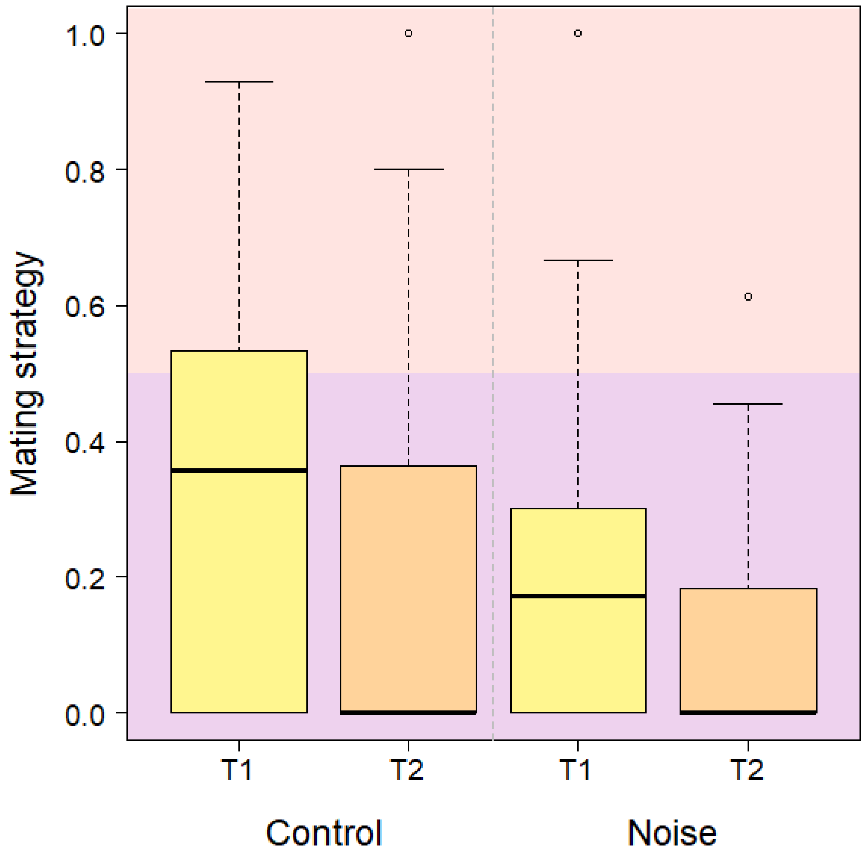
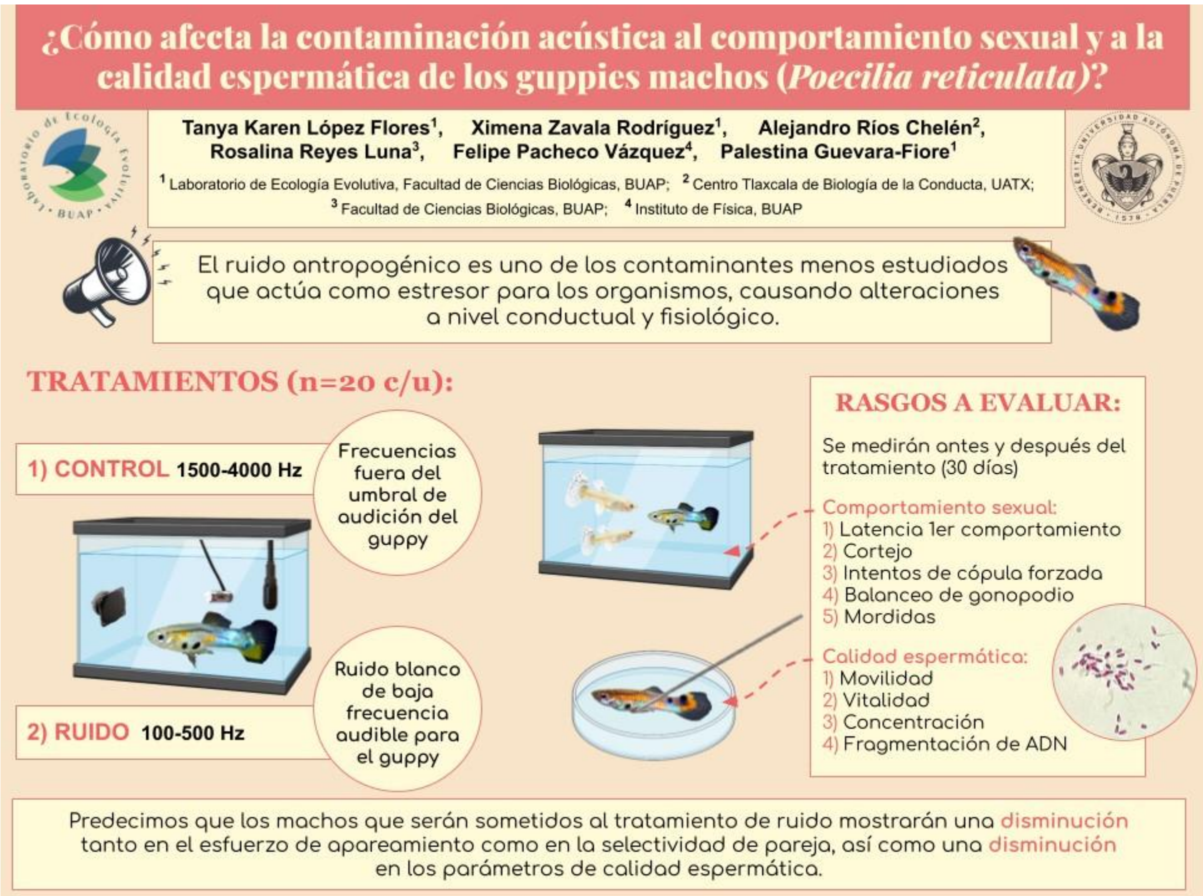


Figure 22. Median ± IQR of the mating strategy of focal males before and after both treatments (control n = 17, noise n = 18), and the 5th and 95th percentiles. Values from 0 to 0.5 (purple colour in the scheme) indicate that the focal male preferred to perform gonopodial thrusts. Values from 0.5 to 1 (pink colour in the scheme) indicate that the focal male preferred to perform sigmoid displays.

APPENDIX 3. Communication of the project

The first protocol (subsequently amended) of this thesis project was presented at the *XXVII International Course of the Biological Bases of Behavior* held from 7 to 9 December 2022 in Morelia, Michoacán, México.



XXVII
INTERNATIONAL COURSE
Biological Bases of Behavior
7 al 9 de diciembre del 2022

SE OTORGA LA PRESENTE CONSTANCIA A:
Tanya Karen López Flores, Ximena Zavala Rodríguez, Alejandro Ariel Ríos-Chelén, Rosalina M. de L. Reyes Luna, Felipe Pacheco Vázquez y Palestina Guevara-Fiore

POR SU PRESENTACIÓN ORAL EN LA SECCIÓN ECOLOGÍA Y EVOLUCIÓN DE LA CONDUCTA

DR. JORGE CONTRERAS GARDUÑO
Organizador

DR. LUIS FELIPE MENDOZA CUENCA
Organizador

Imagen: Anatomy Ambystoma Mexicanum Alexander V. Humboldt / Zoologie

27 AÑOS

A workshop about our model species was held during the *Semana de la Ciencia en el CI 2023* on 15 February 2023 at the Children's Circle in BUAP.

BUAP

La Benemérita Universidad Autónoma de Puebla
A través del Círculo Infantil
otorga el presente

RECONOCIMIENTO

A: **Ximena Zavala Rodríguez**

Por su participación en el evento: **"Semana de la Ciencia en el CI 2023"**
impartiendo el **"Taller de Peces Vivíparos"**
en el que participaron entusiastas todos los alumnos y alumnas de las secciones lactante, maternal y preescolar.

"Pensar bien, para vivir mejor"
H. Puebla de Z., a 15 de febrero de 2023

Mtra. Margarita Trujillo Landa
Directora

A summary of the project was presented at the *Café Científico* organised as part of the celebration of the biology week held on 25 January 2024 in BUAP. Additionally, a poster was created for presentation in the corridors of the Faculty of Biological Sciences.

BENEMÉRITA UNIVERSIDAD AUTÓNOMA DE PUEBLA · 1578

BUAP

Facultad de Ciencias Biológicas
BUAP

La Benemérita Universidad Autónoma de Puebla
A través de la Facultad de Ciencias Biológicas
otorga la presente

CONSTANCIA

A: **Ximena Zavala Rodríguez**

Por su participación en el **Café Científico** presentando el Proyecto:
" Efectos de la contaminación acústica sobre el comportamiento sexual de guppies macho (Poecilia reticulata)"
en el Marco de la Celebración de la Semana de la Biología el día 25 de enero del 2024, de 15:30 a 16:30 hrs, de manera presencial.

Dr. Salvador Galicia Isasmendi
Director de la Facultad de Ciencias Biológicas

"Pensar bien, para vivir mejor"
H. Puebla de Z., a 25 de enero del 2024

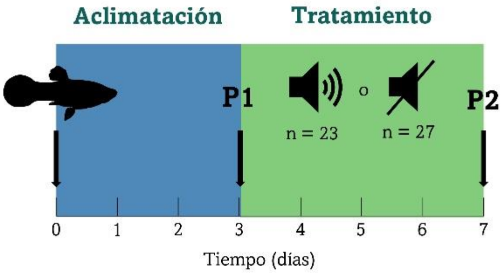
EFECTOS DE LA CONTAMINACIÓN ACÚSTICA SOBRE EL COMPORTAMIENTO SEXUAL DE GUPPIES MACHO (*Poecilia reticulata*)

Ximena Zavala Rodríguez[1], Alejandro Ariel Ríos-Chelén[2], Palestina Guevara-Fiore[1]
[1]Laboratorio de Ecología Evolutiva, Facultad de Ciencias Biológicas, BUAP;
[2]Centro Tlaxcala de Biología de la Conducta, UATX

El ruido generado por el hombre se ha vuelto un elemento cada vez más invasivo en el ambiente, llegando a desencadenar cambios fisiológicos y conductuales en los organismos.

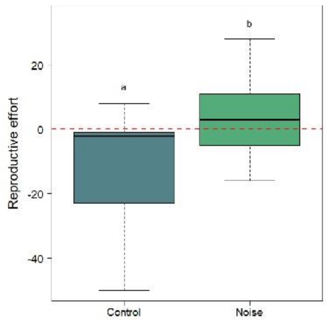
¿Cuáles son los efectos duraderos del ruido blanco artificial sobre el comportamiento sexual de guppies macho?

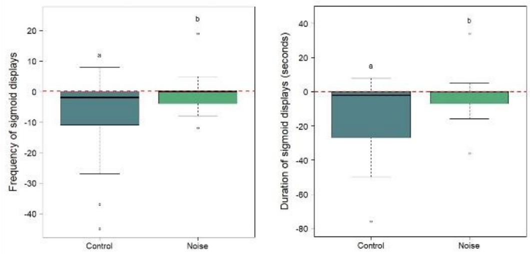
METODOLOGÍA



Pruebas de nado libre (P1 y P2)

RESULTADOS





Incremento en el esfuerzo reproductivo, pero frecuencia y duración de cortejos permanecieron constantes.

Contrario a mis predicciones, los guppies macho invierten más en reproducirse, tras ser expuestos a ruido.

Muchas gracias a Tanya López, Escarlet González, y al Dr. Felipe Pacheco por su valiosa colaboración en este proyecto.