

RESEARCH ARTICLE

Noise-induced hearing loss correlates with inner ear hair cell decrease in larval zebrafish

Rafael A. Lara^{1,2}, Lukas Breitzler¹, Ieng Hou Lau¹, Flora Gordillo-Martinez¹, Fangyi Chen³, Paulo J. Fonseca⁴, Andrew H. Bass⁵ and Raquel O. Vasconcelos^{1,*}

ABSTRACT

Anthropogenic noise can be hazardous for the auditory system and wellbeing of animals, including humans. However, very limited information is known on how this global environmental pollutant affects auditory function and inner ear sensory receptors in early ontogeny. The zebrafish (*Danio rerio*) is a valuable model in hearing research, including investigations of developmental processes of the vertebrate inner ear. We tested the effects of chronic exposure to white noise in larval zebrafish on inner ear saccular sensitivity and morphology at 3 and 5 days post-fertilization (dpf), as well as on auditory-evoked swimming responses using the prepulse inhibition (PPI) paradigm at 5 dpf. Noise-exposed larvae showed a significant increase in microphonic potential thresholds at low frequencies, 100 and 200 Hz, while the PPI revealed a hypersensitization effect and a similar threshold shift at 200 Hz. Auditory sensitivity changes were accompanied by a decrease in saccular hair cell number and epithelium area. In aggregate, the results reveal noise-induced effects on inner ear structure–function in a larval fish paralleled by a decrease in auditory-evoked sensorimotor responses. More broadly, this study highlights the importance of investigating the impact of environmental noise on early development of sensory and behavioural responsiveness to acoustic stimuli.

KEY WORDS: Noise pollution, Auditory threshold shifts, Saccule, Hypersensitization, Prepulse inhibition

INTRODUCTION

Increasing levels of noise pollution are considered a potential threat to the auditory system and overall physiological condition of animals, including humans (Hammer et al., 2014; World Health Organization, 2015; Peris, 2020). Overexposure to elevated sound levels may affect inner ear sensory receptors, resulting in neuropathy and/or cell death and leading to temporary or permanent noise-induced hearing loss (NIHL) (Hu et al., 2002; Kurabi et al., 2017; Zheng and Zuo, 2017). The effects of acoustic trauma on the auditory periphery can induce, in turn, changes in the central auditory system at morphological, physiological and functional levels (Wang et al., 2002; Eggermont, 2015). Impaired

auditory function due to noise exposure may also result in changes in sensorimotor behaviours. For instance, Hickox and Liberman (2014) reported that mice exposed to 94–100 dB re. 20 μ Pa noise for 2 h showed increased thresholds in acoustic startle responses, prepulse inhibition and activation of auditory processing along with behavioural hyperactivity.

Increasing evidence shows that the molecular and cellular mechanisms associated with NIHL are similar to those described for age-related and drug-induced hearing loss, although recent investigations also suggest that the different types of acquired hearing loss might differ in cell death signalling and homeostatic pathways (Wong and Ryan, 2015; Yang et al., 2015). Overall, there is a substantial lack of information on the onset and progression of noise-induced hair cell degeneration, as well as on the mechanisms of synaptopathy and recovery. Although neurotrophins have shown promising regenerative functions after acoustic trauma, more research is needed on potential protective targets and therapeutic agents (Ton and Parng, 2005; Le Prell et al., 2007).

Although mammals have long been used to investigate how noise impacts the auditory system (Ketten, 1992; Ketten, 1998; Rabin et al., 2006; Kujawa and Liberman, 2009; Valero et al., 2017), zebrafish (*Danio rerio*) have become an important model to investigate the mechanisms of inner ear development and hair cell regeneration, and to screen for ototoxicity (Brignull et al., 2010; Stawicki et al., 2015; Wang et al., 2017; Breitzler et al., 2020). Larval zebrafish at 5 days post-fertilization (dpf) possess a functioning auditory system with processing pathways of auditory information (the medial octavolateral nucleus, torus semicircularis, medial hindbrain and thalamus) that overall resemble those found in adult fish and mammals (Vanwallegheem et al., 2017). Moreover, larval zebrafish show a robust acoustic startle response that is easy to quantify (Monroe et al., 2016; Bhandiwad et al., 2018) and is controlled by well-established neural circuitry (Korn and Faber, 2005; Tabor et al., 2014). Based on these features, larval zebrafish are considered a tractable model system that can be used for testing the impact of acoustic trauma on auditory-dependent sensorimotor function and behaviour.

Few studies have evaluated long-term noise effects on animal health (Dooling and Popper, 2007; Alimohammadi et al., 2018; Simmons and Narins, 2018), and even fewer have focused on early critical periods for the development and establishment of adult phenotypic traits (Bureš et al., 2017a; Dorado-Correa et al., 2018; Mueller, 2018; Erbe et al., 2019; Lara and Vasconcelos, 2021). Compared with other vertebrates, especially birds and mammals (Perry, 1998; Dooling and Popper, 2007; Ketten, 2008, 2012; Ortega et al., 2012; Erbe et al., 2018), the relationship between inner ear structure and auditory function following acoustic trauma has been scarcely examined in fishes (Scholik and Yan, 2001; Smith et al., 2004, 2006). To our knowledge, there is no information on the effects of noise exposure on the inner ear and associated hearing loss

¹Institute of Science and Environment, University of Saint Joseph, Macao S.A.R., China. ²Departamento de Biología, Universidad de Sevilla, 41012 Sevilla, Spain.

³Department of Biomedical Engineering, South University of Science and Technology of China, Guangdong, China. ⁴Departamento de Biología Animal and cE3c - Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal. ⁵Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA.

*Author for correspondence (raquel.vasconcelos@usj.edu.mo)

 R.A.L., 0000-0002-3239-0854; R.O.V., 0000-0003-1836-7647

in larval fish. This is a particularly important issue to address given that increasing evidence shows that fish rely on acoustic cues from the soundscape to localize suitable habitats for settlement (Simpson et al., 2004; Leis and Lockett, 2005; Montgomery et al., 2006; Vermeij et al., 2010; Parmentier et al., 2015) and that anthropogenic noise may disrupt habitat identification and impair orientation at early life stages (Simpson et al., 2005; Caiger et al., 2012; Holles et al., 2013; Holmes et al., 2017).

In this regard, a recent study by Bhandiwad et al. (2018) evaluated the impact of long-term noise on auditory-evoked startle responses in larval zebrafish at 5–7 dpf. The authors observed significant noise-induced increases in startle response thresholds and hypersensitization to startle-inducing stimuli. These observations, however, were not related to changes in absolute auditory thresholds (determined based on the prepulse inhibition behavioural assay), but were specific to auditory-evoked escape responses.

The goal of the present study was to test the effect of chronic noise exposure on auditory sensitivity of larval zebrafish both through evoked potential recordings from a population of hair cells in the inner ear saccule, which plays a major role in hearing in teleosts (Lu and Desmidt, 2013), and by measuring sensorimotor responses to acoustic stimuli based on the prepulse inhibition (PPI) assay. We further aimed to relate noise-induced sensory loss to potential changes in saccular morphology. We hypothesized that acoustically induced stress would induce hypersensitization (Bhandiwad et al., 2018) and auditory threshold shifts, along with changes in saccular hair cell number (Monroe et al., 2016; Breitzler et al., 2020).

MATERIALS AND METHODS

Zebrafish: husbandry and sampling

Zebrafish, *Danio rerio* (F. Hamilton 1822), eggs were obtained from either wild-type adults (AB line) or Et(krt4:GFP)^{sqet4} (ET4) adults with GFP expression in hair cells (Yao et al., 2016), initially purchased from China Zebrafish Resource Center (CZRC, China) and reared at the research facilities of the University of Saint Joseph, Macao. These two zebrafish lines are known to have equivalent auditory sensitivity and inner ear morphology at the larval stage (Monroe et al., 2016). Stockfish were maintained in a standalone housing system (model AAB-074-AA-A, Yakos65, New Taipei City, Taiwan) with filtered and aerated water (pH 7–8; 400–550 μS conductivity) at $28\pm 1^\circ\text{C}$, under a 12 h:12 h light:dark cycle and acoustic features as described by Lara and Vasconcelos (2019). For each experimental trial, eggs were collected within 2 hours post-fertilization (hpf) from 2–6 breeding tanks, each containing about 10 females and 5 males. Collected eggs were mixed and randomly distributed into 2 groups of 50 each. Each group was allocated to either noise treatment or silent condition (control).

Nine experimental trials (acoustic treatments versus control) were conducted for saccular potential recordings and morphological analysis. For these tests, larval zebrafish were consistently collected between 10:00 h and 11:00 h at two developmental stages, 3 and 5 dpf. These developmental stages were selected because at 3 dpf, embryos already have a functioning inner ear (Lu and Desmidt, 2013) and at 5 dpf, specimens exhibit auditory-evoked escape responses that are affected by previous noise exposure (Bhandiwad et al., 2018). At the end of the experimental trials, specimens were euthanized in 300 mg l^{-1} of tricaine methanesulfonate (MS-222, ThermoFisher Scientific Inc., Waltham, MA, USA) based on Strykowski and Schech (2015). For the PPI assay, only specimens at 5 dpf were used. A total of 14 trials were conducted – 7 trials per

frequency tested (100 and 200 Hz) – with 10 individuals per group. After data collection, individuals were either euthanized (noise-treated group) or returned to stock conditions (control).

All experimental procedures complied with the ethical guidelines enforced at the University of Saint Joseph and approved by the Division of Animal Control and Inspection of the Civic and Municipal Affairs Bureau of Macao (China), licence AL017/DICV/SIS/2016.

Acoustic treatments

Acoustic treatments followed Lara and Vasconcelos (2021) and were carried in glass tanks (60 cm length \times 30 cm width \times 50 cm height) equipped with top built-in illumination ($\sim 7000\text{ lx}$ on a 12 h:12 h light:dark cycle) and covered with a Styrofoam structure to control for light, temperature and noise conditions. No filtering system was used to avoid additional noise, but complete water changes were carried out between trials. The treatment tanks were mounted on top of Styrofoam boards placed over two granite plates (1.5 cm thick) spaced by rubber pads to reduce transmission of external vibrations. Eggs were placed inside a custom-made fine-mesh cylindrical net box (5 cm diameter, 6 cm high) that was suspended $\sim 7\text{ cm}$ above an underwater speaker (UW30, Lubell Labs, Columbus, OH, USA) that rested on top of a sponge in the tank bottom. Speakers were connected to audio amplifiers (Aishang class ST-50 Amplifier, Zhonghe Electronic Equipment Co., Ltd, Hangzhou, China), which were connected to a laptop running Adobe Audition 3.0 for windows (Adobe Systems Inc., San Jose, CA, USA). In the control group, the amplifier connected to the speaker was switched on but without playback, reaching a sound pressure level (SPL) of 103–108 dB re. $1\text{ }\mu\text{Pa}$, LZS – root mean square (RMS) sound level obtained with slow time and linear frequency weightings: 6.3 Hz to 20 kHz.

In the noise treatment group, fish were continuously exposed to white noise at 150 dB re. $1\text{ }\mu\text{Pa}$, an amplitude level representative of freshwater habitats characterized by anthropogenic noise activity such as shipping (Amoser et al., 2004; Codarin et al., 2009) and noisier zebrafish housing systems (Lara and Vasconcelos, 2019). This noise level is also known to affect survivability, induce physiological stress and cause anxiety-like responses in larval zebrafish (Lara and Vasconcelos, 2021). The acoustic playback consisted of white noise low-pass filtered at 1500 Hz and adjusted to compensate for the frequency response of the loudspeaker and the tank acoustic properties using Adobe Audition software tools to deliver a relatively flat spectrum. Noise level was calibrated before treatment so that the intended sound level (LZS, RMS sound level) was reached at the bottom of the net box ($\sim 7\text{ cm}$ distance from the speaker) using a hydrophone (Bruel & Kjaer 8104, Naerum, Denmark; frequency range: 0.1 Hz to 120 kHz; sensitivity: $-205\text{ dB re. }1\text{ V }\mu\text{Pa}^{-1} \pm 3\text{ dB}$) connected to a hand-held sound level meter (Bruel & Kjaer model 2270). Additionally, the acoustic treatments were calibrated with a tri-axial accelerometer (M20-040, frequency range 1–3 kHz, GeoSpectrum Technologies, Dartmouth, NS, Canada) that was placed horizontally with the acoustic centre at about 7 cm from the speaker in the position later occupied by the net box containing the specimens. The sound playbacks generated most energy/particle motion in the vertical axis, reaching $\sim 120\text{ dB re. }1\text{ m s}^{-2}$, which was calculated based on the MATLAB script paPAM (Nedelec et al., 2016).

Inner ear saccular potential recordings

Microphonic potential recordings from the saccule followed procedures initially described by Sisneros (2009) and

Vasconcelos et al. (2011), and adapted to larval zebrafish by Rohmann et al. (2014).

Individual larval zebrafish were paralysed in 20 μl of 1 mg ml⁻¹ α -bungarotoxin (Life Technologies, ThermoFisher Scientific Inc.) in Hank's solution and then mounted laterally embedded in 0.5% agarose on top of a 35 mm microscopy dish, with the otic capsule positioned outside the agarose. The specimen was covered in Hank's solution containing 0.0002% Methylene Blue and the dish was placed on a fixed stage microscope (Axio Examiner A1, Carl Zeiss meditec AG, Jena, Germany) equipped with 10 \times N-Achroplan water immersion objectives. The recording platform rested on top of an air table (Kinetic System, Boston, MA, USA) inside a walk-in soundproof chamber (IAC120A3-53, IAC Acoustics, Dongguan, China), while the remaining audiometry setup was located outside. All recordings were obtained at room temperature (20–23°C).

The stimulus probe consisted of a metal needle with a tip of approximately 50 μm diameter, which was positioned at the posterior edge of the left otic capsule along the posterior edge of the saccular otolith, and provided a linear oscillatory motion along an axis \sim 20 deg off the longitudinal axis of the specimen (Lu and Desmidt, 2013; Yao et al., 2016) (Fig. 1A). The position of the stimulus probe remained consistently the same across all trials. Vibratory stimuli were achieved by driving the probe with a piezoelectric actuator (Piezosystem, Jena, Germany) controlled by a lock-in amplifier (SR830, Stanford Research Systems Inc., Sunnyvale, CA, USA) through custom-written MATLAB software (MathWorks, Inc., Natick, MA, USA) modified after Rohmann and Bass (2011). Stimuli consisted of 500 ms bursts of 100–400 Hz (in 100 Hz increments, presented randomly) followed by an interstimulus interval of 1 s, and were repeated 8 times.

The linear motion of the probe was calibrated under the Zeiss microscope with a high-speed camera (FASTEC-IL5-254, Fastec Imaging, San Diego, CA, USA). The displacement of the probe tip was extracted from the high-speed videos, extrapolated to the X - Y plane to check for oscillatory movement of the probe using a custom-written MATLAB script. This information was used to determine threshold values in dB re. 1 μm based on the actual probe displacement at the threshold level.

Microphonic potentials were recorded with glass microelectrodes (2–8 M Ω) filled with 3 mol l⁻¹ KCl and positioned approximately in the middle of the saccular epithelia. A reference electrode (Ag/AgCl) was placed in the medium at the border of the dish. The recorded signals were preamplified (model 5A, Getting Instruments Inc., San Diego, CA, USA), high-pass filtered and further amplified (SR650, Stanford Research Systems Inc.), then fed into the lock-in amplifier for analog-to-digital conversion and processing, and finally analysed on a desktop computer. At each stimulation frequency, the stimulus amplitude was increased until the mean of the eight microphonic potential responses was greater than 2 s.d. above the mean response to background noise with the lock-in amplifier power set to the minimum. The threshold data were reported as dB relative to the minimum stimulus output of the setup (0.004 V from the lock-in amplifier). The noise recorded with the stimulation set to the minimum was similar to the 'responses' measured if either a dead fish or no fish was placed in the recording dish.

PPI assay

Acoustic startle responses from 5 dpf zebrafish were determined with the apparatus developed by Wang et al. (2017) (Fig. 2). A total of 14

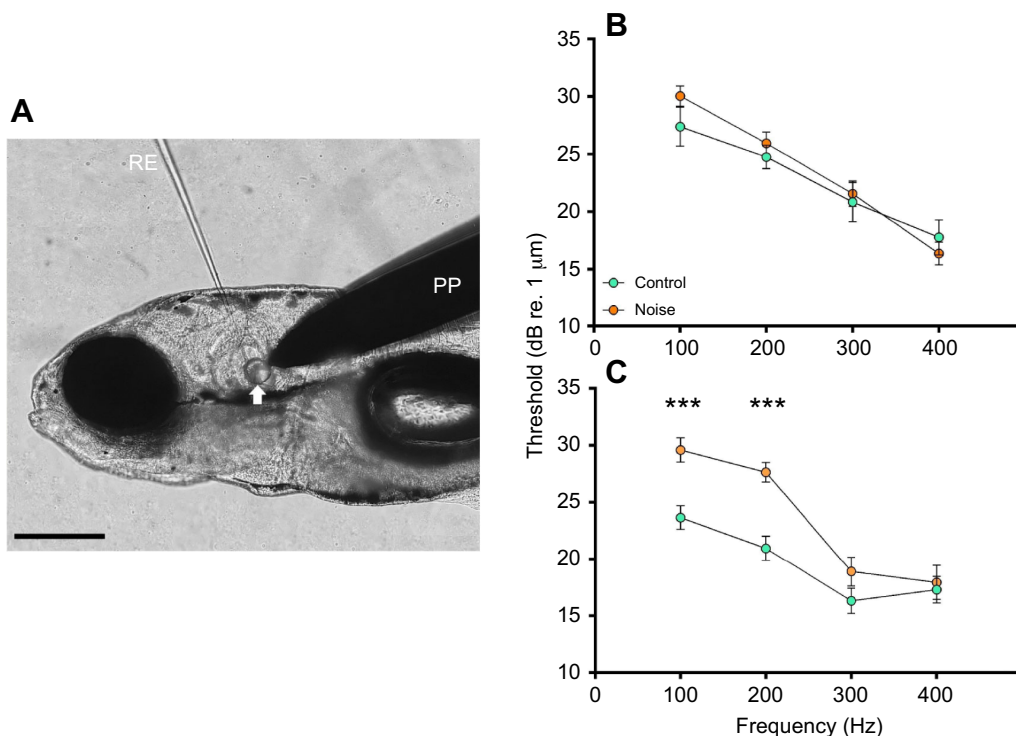


Fig. 1. Microphonic potential recordings from the saccular end organ of the inner ear in zebrafish larvae. (A) Image of a 5 days post-fertilization (dpf) zebrafish mounted in agarose for microphonic potential recordings. Image shows the recording electrode (RE) tip placed underneath the saccular otolith (arrow), and the stimulus probe (PP) touching the posterior edge of the otolith. Scale bar: 200 μm . (B) Microphonic thresholds obtained across the various test frequencies from 3 dpf zebrafish from the control ($N=14$) and noise-treated group ($N=8$). (C) Microphonic thresholds from 5 dpf zebrafish from the control ($N=18$) and noise-treated group ($N=11$), showing significant differences at 100 Hz ($F_{1,22}=17.60$, $***P<0.001$) and 200 Hz ($F_{1,27}=23.84$, $***P<0.001$). Values are means \pm s.e.m.

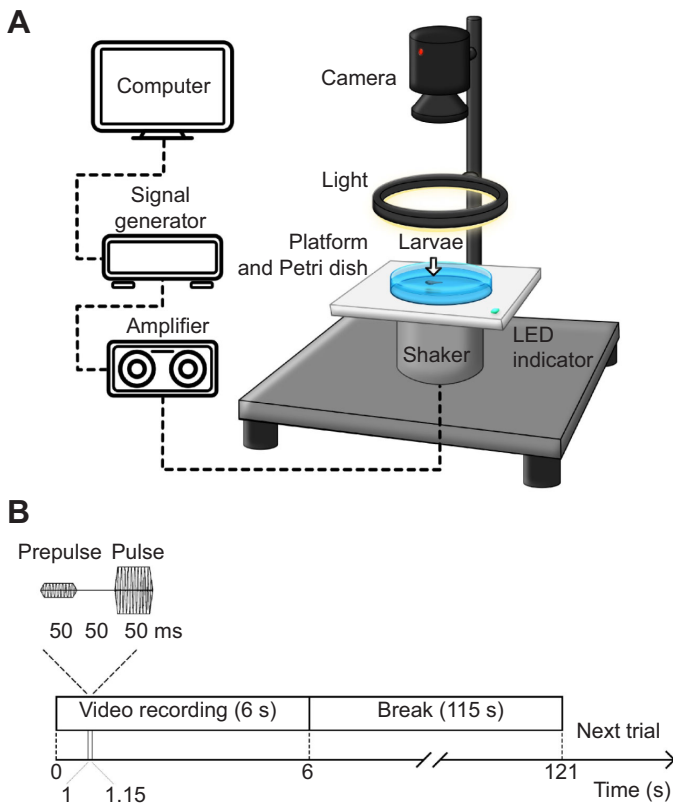


Fig. 2. Prepulse inhibition (PPI) assay. (A) Schematic representation of the setup used to conduct the PPI recordings. (B) Time presentation and recording protocol used in the PPI test.

trials were conducted. Groups of 10 larvae were gently pipetted into a 3D-printed dish platform (8 cm diameter) containing system water (~2 mm depth). The dish was illuminated from above with an LED ring (infrared wavelength at 850 nm, model HA92123, Feiye, Guangzhou, China) and the larvae behaviour was recorded with a digital camera (CS-S6-6C12WFBR, 4 K HD, EZVIZ, Hangzhou, China) that was suspended above of the light ring. The test frequencies and amplitudes were defined using a QT Platform script (The QT Company, Espoo, Finland) that controlled the signal generator (model AUDIO-V1.0.3-20181028, designed by F.C., Southern University of Science and Technology, Guangdong, China) connected to an amplifier (model TPA-2578AY, Weiliang, Foshan, China) that drove a mini vibrator (frequency range: 60 Hz to 20 kHz, model QY50R-Z, Haoshengyuan Inc., Dongguan, China). The particle acceleration at the water surface was initially calibrated with a laser Doppler interferometer (model OFV-505, Polytec GmbH, Baden-Württemberg, Germany) (Wang et al., 2017). Prepulse stimulation consisted of tone bursts of 50 ms at 100 and 200 Hz (frequencies that previously showed differences in microphonic potential recordings), and varying particle acceleration levels ($-\infty$, -35 , -30 , -25 , -20 and -15 dB re. 1 m s^{-2}). The startle responses were induced with pure tones of 50 ms at the same frequencies as the prepulse stimulus but at 29 and 25 dB re. 1 m s^{-2} for 100 and 200 Hz, respectively, and delivered after a 50 ms interval (Fig. 2B). Stimulation was repeated 10 times at each level with 120 s intervals between presentations to avoid habituation. Six-second videos ($120 \text{ frames s}^{-1}$, 8.3 ms per frame, 0.707 mm per pixel) were recorded per prepulse stimulus level and the average swimming velocity was tracked and calculated from each individual fish's displacement in the X - Y coordinates by subtracting sequential frames

of the video recording (Wang et al., 2017). For each treatment group, the startle responses (quantified as swimming velocity) to successive and increasing prepulse amplitudes allowed determination of the amplitude level that caused a significant decrease in the motor response (PPI). This prepulse amplitude level was compared between treatment groups (see 'Statistical analysis', below).

Potential differences in startle responses could derive from differences in swimming patterns and motor abilities; thus, a separate set of 15 specimens from each noise exposure and control group were recorded for 10 min in an open field consisting of a Petri dish (equivalent size to the PPI dish) at 28°C in a DanioVision chamber (Noldus Technologies, Wageningen, The Netherlands). A total of 9 open field recordings were conducted. Videos were analysed using Ethovision XT (Noldus Technologies) and total distance moved and time spent moving were measured for each group.

Inner ear saccule analysis

The inner ear saccular hair cell bundles of larval ET4 that were subject to noise versus control conditions were morphologically analysed. After euthanasia, specimens were immediately fixed in 4% paraformaldehyde (PFA) at 4°C overnight. The following day, samples were rinsed 3 times for 10 min in PBS. To visualize the entire saccular epithelia, the otoliths from 3 and 5 dpf larvae were dissolved in 1% or 2% Triton X-100 (Sigma-Aldrich, St Louis, MO, USA), respectively, for up to 24 h at 4°C. Samples were subsequently rinsed in PBS and then mounted laterally on microscope slides containing squared holes previously prepared with vinyl tape and containing Vectashield anti-fading solution (Vector Laboratories, Burlingame, CA, USA).

Samples were visualized under a confocal laser scanning microscope (Stellaris 8, Leica Microsystems, Buffalo Grove, IL, USA) with a 488 nm laser line (Leica Microsystems, Wetzlar, Germany). Imaging was based on a z -stack of 45 images (spanning approximately 181 μm , 4 μm per image) and 3D reconstruction analysis was performed using Leica LAS X 3.0.14 software (Leica Microsystems). Quantification of saccular hair cell number was made by counting the hair cell bundles in the whole epithelia and the epithelial area was measured using Leica LAS X 3.0.14 software.

Statistical analysis

Differences in inner ear sensitivity based on saccular microphonics between the two developmental stages, and between noise-exposed versus control groups, were tested with repeated measures ANOVA, using noise or age as a between-subject factor, while the different frequencies were the repeated measures (within-subject factor). Differences at specific frequencies were further verified based on one-way ANOVA.

Differences in PPI responses between treatments were also determined based on repeated measures ANOVA, with noise as a between-subject factor and prepulse amplitude as repeated measures. Only the trials that revealed a significant decrease in response to increasing prepulse amplitude (PPI) were considered for the analysis, which was first verified based on one-way ANOVA for each group and frequency separately.

The variables related to larval behavioural patterns, i.e. total swimming distance and time spent moving, were compared with one-way ANOVA tests between treatment and control groups. Comparison of the inner ear morphological features (hair cell number and epithelial area) was also carried out with one-way ANOVA.

ANOVA were followed by LSD multiple comparison *post hoc* tests to check for pairwise differences. Parametric tests were used as

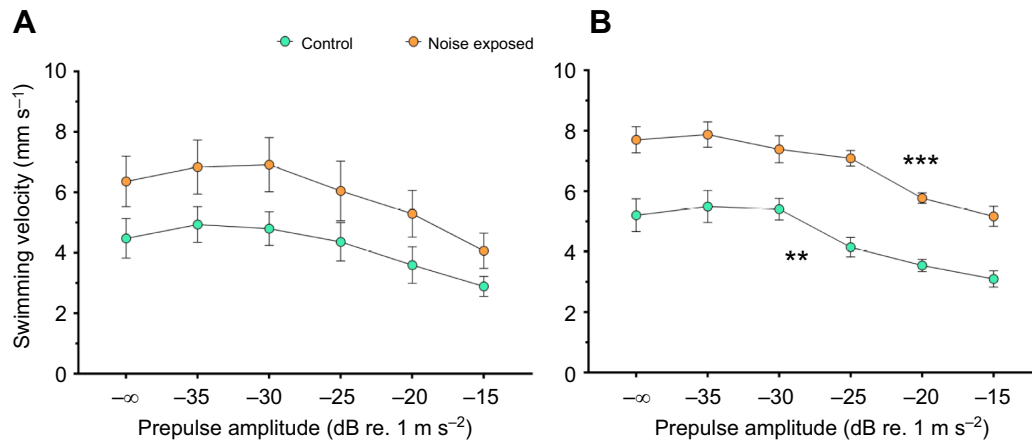


Fig. 3. Auditory-evoked swimming responses to 100 and 200 Hz stimulus. Data are presented as mean \pm s.e.m. swimming velocity in response to various prepulse amplitudes for control and noise-treated larvae ($N=20$ per trial, 5 dpf). (A) At 100 Hz, no significant changes in response thresholds were observed. (B) At 200 Hz, a significant decline in responsiveness was found in the control group between -30 and -25 dB re. 1 m s^{-2} ($F_{1,38}=8.21$, $**P=0.006$), while in the noise-treated group, the decline was only found between -25 and -20 dB re. 1 m s^{-2} ($F_{1,38}=4.93$, $***P<0.001$).

data were normally distributed and variances were homogeneous. Assumptions for parametric analyses were confirmed through the inspection of normal probability plots and by the Levene test. Statistical analyses were performed using SPSS v26 (IBM Corp. Armonk, NY, USA).

RESULTS

Inner ear saccular hair cell sensitivity

Microphonic potentials were recorded from saccular hair cells in both 3 and 5 dpf larvae (Fig. 1B,C) and displayed an age-related enhancement in sensitivity of up to 4 dB (at 100 and 200 Hz). The microphonic threshold for control groups at 3 dpf decreased from 27 ± 4 to 18 ± 5 dB re. $1 \mu\text{m}$ (means \pm s.e.m.) from 100 to 400 Hz. At 5 dpf, the threshold decreased from 23 ± 4 to 17 ± 4 dB re. $1 \mu\text{m}$ at the same frequencies. Significant differences in auditory thresholds were found between these two developmental stages ($F_{1,24}=10.05$, $P=0.004$).

Noise treatment did not cause significant changes in the microphonic response at 3 dpf ($F_{1,10}=0.31$, $P>0.05$; Fig. 1B), but a noise-induced sensitivity loss was found at 5 dpf ($F_{1,12}=8.18$, $P<0.001$; Fig. 1C). Increased thresholds ranged from 30 ± 3 to 16 ± 3 dB re. $1 \mu\text{m}$ at 3 dpf, between 100 and 400 Hz. At 5 dpf, they ranged from 29 ± 3 to 18 ± 5 dB re. $1 \mu\text{m}$ (between 100 and 400 Hz). The significant differences found at 5 dpf were identified at both 100 Hz ($F_{1,22}=17.60$, $P<0.001$) and 200 Hz ($F_{1,27}=23.84$, $P<0.001$), with threshold shifts of up to 6 and 7 dB re. $1 \mu\text{m}$, respectively.

Auditory-evoked sensorimotor responses

Noise exposure induced a significant increase in swimming velocity from $3.56\pm 0.19 \text{ mm s}^{-1}$ (control; mean \pm s.e.m.) to $5.26\pm 0.21 \text{ mm s}^{-1}$ (noise exposed) at 100 Hz, and from $4.34\pm 0.24 \text{ mm s}^{-1}$ (control) to $7.54\pm 0.21 \text{ mm s}^{-1}$ (noise exposed) at 200 Hz, corresponding to increments of 34.9% and 60.9%, respectively (Fig. 3). This hypersensitization was significant at both

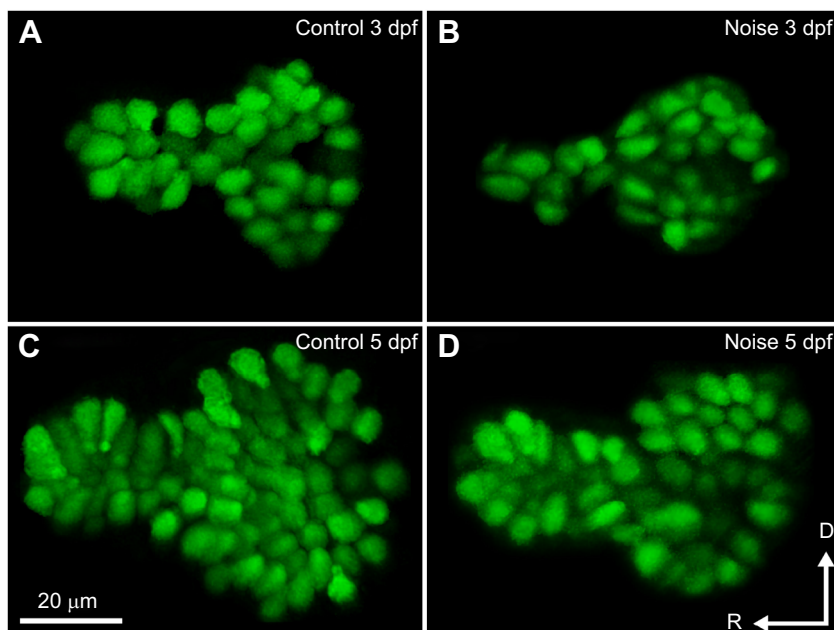


Fig. 4. Inner ear saccular morphology. Representative confocal images of whole saccular sensory epithelia from Et(krt4:GFP)sqet4 (ET4) zebrafish larvae, expressing GFP in hair cells. (A,B) Images of 3 dpf (A) control and (B) noise-treated larvae. (C,D) Images of 5 dpf (C) control and (D) noise-treated larvae. The background was obscured for visual clarity. Scale bar: $20 \mu\text{m}$. R, rostral; D, dorsal.

test frequencies (100 Hz: $F_{1,30}=21.36$, $P<0.001$; Fig. 3A; 200 Hz: $F_{1,37}=25.00$, $P<0.001$; Fig. 3B).

Comparison of the startle response across increasing prepulse amplitudes (PPI) revealed auditory sensitivity loss associated with noise exposure. At 200 Hz, the startle response significantly decreased between -30 and -25 dB re. 1 m s^{-2} prepulse amplitude for the control group ($F_{1,38}=8.21$, $P=0.006$), in contrast to the noise-treated specimens, which showed a significant reduction only between -25 and -20 dB re. 1 m s^{-2} ($F_{1,38}=4.93$, $P<0.001$) (Fig. 3B). These results indicate a noise-induced 5 dB shift in response threshold at 200 Hz. However, at 100 Hz, there was no significant change in swimming response in either the control ($F_{1,33}=0.28$, $P>0.05$) or the noise-treated group ($F_{1,37}=1.47$, $P>0.05$) (Fig. 3A).

Additionally, to test whether larval general locomotor activity was affected by the acoustic treatment, specimens were observed in an open field arena. Noise-exposed larvae showed significantly lower swimming speed ($F_{1,191}=14.25$, $P<0.001$) and time spent swimming ($F_{1,191}=1.829$, $P<0.001$), suggesting additional alterations in their locomotor capabilities.

Inner ear saccular morphology

In order to evaluate whether auditory sensitivity changes were associated with differences in inner ear morphology, we investigated saccular hair cell number and epithelial area of 3 and 5 dpf zebrafish exposed to the aforementioned conditions (Fig. 4).

The number of saccular hair cells increased significantly with age both in control (from 49 to 65, a 33% increment; $F_{1,32}=38.23$, $P<0.001$) and noise-exposed (from 38 to 53, a 39% increment; $F_{1,37}=22.19$, $P<0.001$) groups. Although the general shape of the epithelia did not change, acoustic treatment caused a significant reduction in number at 3 dpf (~ 10 – 11 fewer bundles, 21% reduction; $F_{1,39}=14.16$, $P<0.001$) and at 5 dpf (12 – 13 fewer bundles, 19% reduction; $F_{1,30}=19.16$, $P<0.001$) (Fig. 5A). Additionally, saccular epithelial area also decreased 23% at 3 dpf ($F_{1,19}=4.71$, $P=0.044$) and 35% at 5 dpf ($F_{1,19}=18.19$, $P<0.001$) (Fig. 5B).

Consistent with the parallel changes in hair cell number and epithelia area, hair cell density did not reveal differences between noise-treated and control groups (3 dpf: $F_{1,19}=2.56$, $P>0.05$; 5 dpf: $F_{1,19}=3.19$, $P>0.05$). Finally, there were no age-related differences in hair cell number and epithelial growth between the two experimental groups ($F_{1,19}=4.03$, $P>0.05$).

DISCUSSION

To our knowledge, the present study provides the first evidence that exposure to increased noise levels can impact auditory sensitivity

and the amount of auditory hair cell receptors in larval fish, and that such sensory loss correlates with a behavioural hypersensitization. We used larval zebrafish (*Danio rerio*) as our model system, showing that this species can be used to evaluate the impact of noise on auditory function in early ontogeny.

Noise-induced changes in inner ear function and structure

We assessed inner ear saccular sensitivity in 3 and 5 dpf zebrafish larvae based on microphonic potential recordings, a reliable method to assess peripheral auditory function in zebrafish at these developmental stages (Lu and Desmidt, 2013; Rohmann et al., 2014), when the auditory pathways are already functional (Tanimoto et al., 2011; Vanwalleghem et al., 2017).

The results demonstrate an age-related enhancement in saccular sensitivity of up to 4 dB (at 100 and 200 Hz) accompanied by a 33% increase in hair cell number. These findings are similar to those of Lu and DeSmidt (2013) and Yao et al. (2016), who showed an improvement of 8 and 4 dB at 100 and 200 Hz, respectively, along with a 34% hair cell increment between 3 and 5 dpf.

We showed that chronic exposure to elevated noise levels (150 dB re. $1 \mu\text{Pa}$, white noise) causes hearing loss of up to 6–7 dB in larval zebrafish at 5 dpf. The lack of noise-induced threshold shifts at 3 dpf might be related to differences in hair cell sensitivity, inner ear development and/or the overall duration of the acoustic treatment. A few studies have identified noise-induced auditory threshold shifts in fish species at the adult stage (Scholik and Yan, 2001; Amoser and Ladich, 2003; Smith et al., 2004; Popper et al., 2005), including zebrafish (Breitzler et al., 2020). But these studies typically relied on auditory evoked potential recordings that measure overall sensitivity of both peripheral and central auditory pathways, showing threshold increases of up to 30 dB re. $1 \mu\text{Pa}$. Here, we investigated the impact of the acoustic environment on sensitivity at the sensory receptor level of the inner ear saccule, which is considered to serve mainly a hearing function in most teleosts (Popper and Fay, 1973, 1993; Schuck and Smith, 2009).

In order to evaluate whether differences in the saccular sensitivity were related to changes in the sensory epithelia, we investigated a possible noise-induced effect on hair cell number. Noise exposure induced a 21% and 19% decrease in saccular hair cell number at 3 and 5 dpf, respectively. Such changes were not related to changing hair cell density, but to an overall reduction in sensory epithelial size (23% and 35% reduction in total area at 3 and 5 dpf, respectively). Similarly, Uribe et al. (2018) found saccular and lateral line hair cell damage in 6 dpf zebrafish induced by underwater cavitation producing high intensity broadband sounds. According to these authors, acoustic exposure to circa 186 dB re. $1 \mu\text{Pa}$ RMS for

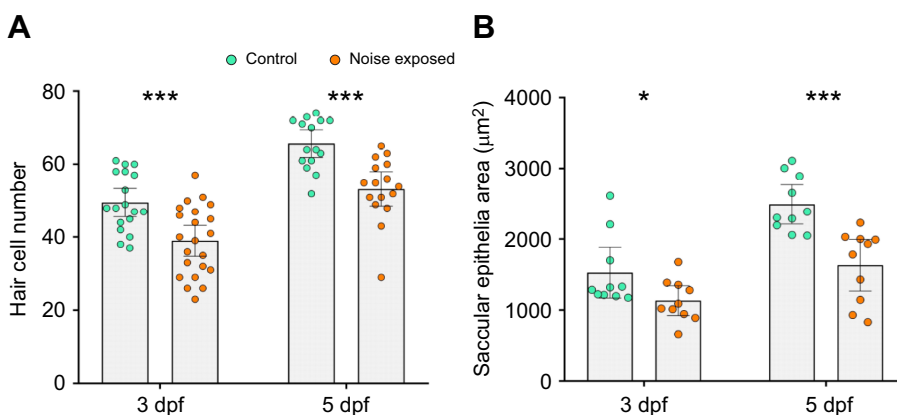


Fig. 5. Hair cell number and saccular epithelia area. (A) Comparison of the number of saccular hair cells between control and noise-exposed larval zebrafish at 3 dpf ($F_{1,39}=14.16$, $***P<0.001$) and 5 dpf ($F_{1,30}=19.16$, $***P<0.001$). (B) Comparison of the saccular epithelia area between control and noise-exposed larval zebrafish at 3 dpf ($F_{1,19}=4.71$, $*P=0.044$) and 5 dpf ($F_{1,19}=18.19$, $***P<0.001$). Values are means \pm 95% confidence intervals.

120 min reduced the number of saccular hair cells by 14% and lateral line hair cells by 30%, which was recovered 72 h post-exposure. Our findings show that noise exposure affects inner ear development, potentially damaging hair cells in larval zebrafish, when accessory hearing structures are not yet present and auditory stimulation most likely comes from particle motion (Grande and Young, 2004). Future studies should evaluate the potential impact of noise on otolith development and the integrity of the otolith membrane, and quantify hair cell death and damage.

Schuck and Smith (2009) and Breitzler et al. (2020) reported noise-induced damage of saccular hair cells in adult zebrafish. Exposure to 100 Hz pure tone at 179 dB re. 1 μ Pa for 36 h resulted in 43% hair cell loss in the posterior region of the saccule (Schuck and Smith, 2009), whereas white noise treatment at 150 dB re. 1 μ Pa for 24 h caused 15% hair cell loss in adult zebrafish. Other studies have also investigated the impact of acoustic trauma on the inner ear of adult fish from various species and reported noise level-dependent saccular hair cell loss (Schuck and Smith, 2009; Monroe et al., 2015).

Only a few studies have evaluated the effect of chronic noise treatment in early ontogeny (Bureš et al., 2017a,b; Dorado-Correa et al., 2018), which is a critical window for the establishment of phenotypic traits (Mueller, 2018). Bureš et al. (2017a) reported frequency-dependent neuronal alterations in sound intensity representation in adult rats (Long–Evans strain) that were briefly exposed to noise (up to 80 dB SPL) in early ontogeny, while Dorado-Correa et al. (2018) reported faster telomere loss in juvenile zebra finches (*Taeniopygia guttata*) exposed to traffic noise (65 and 85 dB re. 20 μ Pa). To our knowledge, the present work is the first study reporting the impact of the acoustic environment on the inner ear and associated sensitivity loss in a larval fish.

Fishes have evolved to enhance sound reception in aquatic habitats within local environmental constraints (Amorim et al., 2018). By listening to the aquatic background noise, fish can extract critical biotic information about the presence of conspecifics and heterospecifics, and perceive important abiotic information for orientation (Popper and Fay, 1993; Lagardere et al., 1994; Ladich and Schulz-mirbach, 2013). More specifically, larval fish undergo auditory sensitivity improvements during growth (Vasconcelos et al., 2015) and rely on acoustic cues to detect suitable habitats for settlement, and the presence of anthropogenic noise may interfere with their hearing sense, habitat identification and impair orientation (Simpson et al., 2004; Leis and Lockett, 2005; Montgomery et al., 2006; Parmentier et al., 2015).

Noise is known to cause impaired growth and development in early ontogeny (de Soto et al., 2013), tissue damage in the inner ear (Casper et al., 2013) and molecular and cellular changes along the auditory pathway (Lim, 1986; Bohne et al., 2007; Yang et al., 2015; Mancera et al., 2017), which may also have contributed to the decreased saccular sensitivity observed in our study. The molecular mechanisms underlying NIHL and their impact on inner ear structure–function remain to be fully explored in this model species.

Noise effects on acoustic startle responses

We additionally investigated the effects of acoustic overexposure on the sensory-motor response to acoustic stimuli in larval zebrafish at 5 dpf. We found that continuous exposure to 150 dB re. 1 μ Pa white noise induced a generalized hypersensitization of the acoustic startle response, as observed by a significant increase in swimming velocity (up to 41%). Such hypersensitization was apparently not influenced by an alteration in total locomotor activity as the

swimming speed and the time spent swimming were actually lower in the noise-treatment group than in the control group.

The hypersensitization observed in this study is similar to the startle-inducing hypersensitization noted in prior studies using fish species (Purser and Radford, 2011; Bhandiwad et al., 2018). Purser and Radford (2011) exposed adult three-spined stickleback (*Gasterosteus aculeatus*) to recreational boat noise conditions (white noise, 100–1000 Hz at 128 dB SPL) and identified a twofold increase in the number of startle responses to broadband stimuli. Bhandiwad et al. (2018) exposed 5–7 dpf larval zebrafish to white noise at 20 dB re. 1 m s⁻² using a one-dimensional shaker and also found significant hypersensitization of startle responses in noise-treated specimens. Similar hypersensitivity was also observed in rodent species (hamster LVG strain and CBA/CaJ male mice) after acoustic overexposure (Chen et al., 2013; Hickox and Liberman, 2014), suggesting that noise-induced sensitization of sensory-motor responses might be a common effect among vertebrates.

We report that noise caused a significant increase in absolute PPI thresholds in larval zebrafish at 200 Hz. PPI is thought to be regulated by GABAergic and glycinergic interneurons in the zebrafish hindbrain that inhibit the firing activity of Mauthner cells and receive direct input from primary VIIIth nerve afferents (Weiss et al., 2008). Noise exposure could potentially affect this neuronal pathway (Bhandiwad et al., 2018), yet the results presented here suggest that sensitivity loss is probably related to a decreased number of the hair cells and cell damage, but this requires further confirmation.

Bhandiwad et al. (2018) also tested the effect of noise on auditory sensitivity in larval zebrafish at 5–7 dpf, but did not report significant noise-induced changes in absolute PPI threshold. However, these authors used lower stimulation frequencies, 30 and 90 Hz, and, although not significant, the average absolute thresholds were higher in the noise-treated group. Studies using the PPI methodology to test the effects of noise treatment on acoustic startle responses have shown different results, ranging from an increased response magnitude (Hickox and Liberman, 2014; Wang et al., 2017) to a small or even reduced response magnitude of the acoustic startle reflex (Weiss et al., 2008).

We report noise-induced hearing loss using both electrophysiology recordings and behavioural tests in a larval fish, with both experimental approaches identifying a significant increase in auditory threshold at 200 Hz. The lack of significant changes at 100 Hz using the PPI assay is similar to the findings reported at even lower frequencies by Bhandiwad et al. (2018). The difference between the results obtained with the two audiometry systems might be related to limitations of the PPI setup that induced higher variability in acoustic startle responses and less overall inhibition at ≤ 100 Hz, as well as to differences that are specific to the type of response measured (peripheral sensory versus behavioural).

Further research is necessary to confirm the causes of sensory loss at the physiological and behavioural levels. The present work strongly suggests that the zebrafish is a tractable model to investigate noise-induced perceptual disorders, and that underlying changes in the auditory system and behaviour related to acoustic trauma might be conserved across vertebrates. Furthermore, given the extended use of zebrafish in biomedical research, including hearing studies, and considering that this species is typically raised under noisy captive conditions (Lara and Vasconcelos, 2019), our results also highlight the potential interference of the acoustic conditions on the sensory-cognitive development of this model system. Previously, we showed that larval zebrafish subject to the same noise treatment (150 dB white noise) presented heightened physiological stress,

anxiety-related behaviour and impaired spontaneous alternation behaviour (Lara and Vasconcelos, 2021). We now show that noise conditions may affect the detection of auditory stimuli at a receptor cell level with potential consequences for environmental sensory adaptation. Future studies should investigate this phenomenon in natural fish populations and the ecological and evolutionary implications. Further research should also exploit the technical advantages of zebrafish to investigate whether noise exposure in early ontogeny has carryover effects to subsequent life stages and transgenerational consequences.

Acknowledgements

We are thankful to Peng Sun (Southern University of Science and Technology, Guangdong, China) for assistance with the PPI setup and data analysis, and to Alexandre Lebel (University of Saint Joseph, Macao) for the illustration of the PPI setup.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: R.O.V., R.A.L.; Methodology: R.A.L., R.O.V., L.B., F.C., P.J.F., A.H.B.; Software: R.O.V., L.B., F.C., P.J.F., A.H.B.; Validation: R.A.L., R.O.V.; Data collection: R.A.L., I.H.L., F.G.-M.; Data analysis: R.A.L., I.H.L., R.O.V.; Writing - original draft: R.A.L., R.O.V.; Writing - review & editing: R.A.L., R.O.V., P.J.F., A.H.B.; Supervision: R.O.V.; Funding acquisition: R.O.V.

Funding

This study was supported by Fundo para o Desenvolvimento das Ciências e da Tecnologia (FDCT) Macau (046/2018/A2).

References

- Alimohammadi, I., Ahmadi Kanrash, F., Abolghasemi, J., Afzandeh, H. and Rahmani, K. (2018). Effect of chronic noise exposure on aggressive behavior of automotive industry workers. *Int. J. Occup. Environ. Med.* **9**, 170-175. doi:10.15171/ijoem.2018.1375
- Amorim, M. C. P., Vasconcelos, R. O., Bolgan, M., Pedroso, S. S. and Fonseca, P. J. (2018). Acoustic communication in marine shallow waters: Testing the acoustic adaptive hypothesis in sand gobies. *J. Exp. Biol.* **221**. doi:10.1242/jeb.183681
- Amoser, S. and Ladich, F. (2003). Diversity in noise-induced temporary hearing loss in otopharyngeal fishes. *J. Acoust. Soc. Am.* **113**, 2170-2179. doi:10.1121/1.1557212
- Amoser, S., Wysocki, L. E. and Ladich, F. (2004). Noise emission during the first powerboat race in an Alpine lake and potential impact on fish communities. *J. Acoust. Soc. Am.* **116**, 3789-3797. doi:10.1121/1.1808219
- Bhandiwad, A. A., Raible, D. W., Rubel, E. W. and Sisneros, J. A. (2018). Noise-induced hypersensitization of the acoustic startle response in larval zebrafish. *J. Assoc. Res. Otolaryngol.* **19**, 741-752. doi:10.1007/s10162-018-00685-0
- Bohne, B. A., Harding, G. W. and Lee, S. C. (2007). Death pathways in noise-damaged outer hair cells. *Hear. Res.* **223**, 61-70. doi:10.1016/j.heares.2006.10.004
- Breitler, L., Lau, I. H., Fonseca, P. J. and Vasconcelos, R. O. (2020). Noise-induced hearing loss in zebrafish: Investigating structural and functional inner ear damage and recovery. *Hear. Res.* **391**, 107952. doi:10.1016/j.heares.2020.107952
- Brignull, H. R., Raible, D. W. and Stone, J. S. (2010). Feathers and Fins: non-mammalian models for hair cell regeneration. *Brain*, 12-23. doi:10.1016/j.brainres.2009.02.028. Feathers
- Bureš, Z., Popelář, J. and Syka, J. (2017a). Noise exposure during early development impairs the processing of sound intensity in adult rats. *Hear. Res.* **352**, 1-11.
- Bureš, Z., Popelář, J. and Syka, J. (2017b). The effect of noise exposure during the developmental period on the function of the auditory system. *Hear. Res.* **352**, 1-11. doi:10.1016/j.heares.2016.03.008
- Caiger, P. E., Montgomery, J. C. and Radford, C. A. (2012). Chronic low-intensity noise exposure affects the hearing thresholds of juvenile snapper. *Mar. Ecol. Prog. Ser.* **466**, 225-232. doi:10.3354/meps09933
- Casper, B. M., Smith, M. E., Halvorsen, M. B., Sun, H., Carlson, T. J. and Popper, A. N. (2013). Effects of exposure to pile driving sounds on fish inner ear tissues. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **166**, 352-360. doi:10.1016/j.cbpa.2013.07.008
- Chen, G., Lee, C., Sandridge, S. A., Butler, H. M., Manzoor, N. F. and Kaltenbach, J. A. (2013). Behavioral evidence for possible simultaneous induction of hyperacusis and tinnitus following intense sound exposure. *J. Assoc. Res. Otolaryngol.* **14**, 413-424. doi:10.1007/s10162-013-0375-2
- Codarin, A., Wysocki, L. E., Ladich, F. and Picciulin, M. (2009). Effects of ambient and boat noise on hearing and communication in three fish species living in a marine protected area (Miramare, Italy). *Mar. Pollut. Bull.* **58**, 1880-1887. doi:10.1016/j.marpolbul.2009.07.011
- De Soto, N. A., Delorme, N., Atkins, J., Howard, S., Williams, J. and Johnson, M. (2013). Anthropogenic noise causes body malformations and delays development in marine larvae. *Sci. Rep.* **3**, 2831. doi:10.1038/srep02831
- Dooling, R. J. and Popper, A. N. (2007). The effects of highway noise on birds. *Environ. Bioacoustics* **27**, 1-74. doi:10.1121/2.0000244
- Dorado-Correa, A. M., Zollinger, S. A., Heidinger, B. and Brumm, H. (2018). Timing matters: traffic noise accelerates telomere loss rate differently across developmental stages. *Front. Zool.* **15**, 1-8. doi:10.1186/s12983-018-0275-8
- Eggermont, J. J. (2015). The auditory cortex and tinnitus - a review of animal and human studies. *Eur. J. Neurosci.* **41**, 665-676. doi:10.1111/ejn.12759
- Erbe, C., Dähne, M., Gordon, J., Herata, H., Houser, D. S., Koschinski, S., Leaper, R., McCauley, R., Miller, B., Müller, M. et al. (2019). Managing the effects of noise from ship traffic, seismic surveying and construction on marine mammals in Antarctica. *Front. Mar. Sci.* **6**. doi:10.3389/fmars.2019.00647
- Erbe, C., Dunlop, R. and Dolman, S. (2018). Effects of noise on marine mammals. *Effects of Anthropogenic Noise on Animals* **66**, 277-309. doi:10.1007/978-1-4939-8574-6_10
- Grande, T. and Young, B. (2004). The ontogeny and homology of the Weberian apparatus in the zebrafish *Danio rerio* (Ostariophysi: Cypriniformes). *Zool. J. Linn. Soc.* **140**, 241-254. doi:10.1111/j.1096-3642.2003.00097.x
- Hammer, M. S., Swinburn, T. K. and Neitzel, R. L. (2014). Environmental noise pollution in the United States: Developing an effective public health response. *Environ. Health Perspect.* **122**, 115-119. doi:10.1289/ehp.1307272
- Hickox, A. E. and Liberman, M. C. (2014). Is noise-induced cochlear neuropathy key to the generation of hyperacusis or tinnitus? *J. Neurophysiol.* **111**, 552-564. doi:10.1152/jn.00184.2013
- Holles, S. H., Simpson, S. D., Radford, A. N., Berten, L. and Lecchini, D. (2013). Boat noise disrupts orientation behaviour in a coral reef fish. *Mar. Ecol. Prog. Ser.* **485**, 295-300. doi:10.3354/meps10346
- Holmes, L. J., McWilliam, J., Ferrari, M. C. O. and McCormick, M. I. (2017). Juvenile damselfish are affected but desensitize to small motor boat noise. *J. Exp. Mar. Biol. Ecol.* **494**, 63-68. doi:10.1016/j.jembe.2017.05.009
- Hu, B. H., Henderson, D. and Nicotera, T. M. (2002). Involvement of apoptosis in progression of cochlear lesion following exposure to intense noise. *Hear. Res.* **166**, 62-71. doi:10.1016/S0378-5955(02)00286-1
- Ketten, D. R. (1998). Marine mammal auditory systems: A summary of audiometric and anatomical data and its implication for underwater acoustics impacts. *Noaa-NMFS* **62**, 74.
- Ketten, D. R. (1992). The cetacean ear: form frequency and evolution. In *Marine Mammal Sensory Systems* (ed. J. A. Thomas, R. A. Kastelein and A. Ya. Supin), pp. 53-75. Plenum Press.
- Ketten, D. R. (2008). Underwater ears and the physiology of impacts: Comparative liability for hearing loss in sea turtles, birds, and mammals. *Bioacoustics* **17**, 312-315. doi:10.1080/09524622.2008.9753860
- Ketten, D. R. (2012). Marine mammal auditory system noise impacts: evidence and incidence. In *The Effects of Noise on Aquatic Life* (ed. A. N. Popper and A. Hawkins), pp. 207-212. New York, NY: Springer New York.
- Korn, H. and Faber, D. S. (2005). The Mauthner cell half a century later: a neurobiological model for decision-making? *Neuron* **47**, 13-28. doi:10.1016/j.neuron.2005.05.019
- Kujawa, S. G. and Liberman, M. C. (2009). Adding insult to injury: Cochlear nerve degeneration after "temporary" noise-induced hearing loss. *J. Neurosci.* **29**, 14077-14085. doi:10.1523/JNEUROSCI.2845-09.2009
- Kurabi, A., Keithley, E. M., Housley, G. D., Ryan, A. F. and Wong, A. C.-Y. (2017). Cellular mechanisms of noise-induced hearing loss. *Hear. Res.* **349**, 129-137. doi:10.1016/j.heares.2016.11.013
- Ladich, F. and Schulz-Mirbach, T. (2013). Hearing in cichlid fishes under noise conditions. *PLoS ONE* **8**, e57588. doi:10.1371/journal.pone.0057588
- Lagardere, J. P., Bâ@Gout, M. L., Lafaye, J. Y. and Villotte, J. P. (1994). Influence of Wind-Produced Noise on Orientation in the Sole (*Solea-Solea*). *Can. J. Fish. Aquat. Sci.* **51**, 1258-1264. doi:10.1139/f94-125
- Lara, R. A. and Vasconcelos, R. O. (2019). Characterization of the natural soundscape of zebrafish and comparison with the captive noise conditions. *Zebrafish* **16**, 1-13. doi:10.1089/zeb.2018.1654
- Lara, R. A. and Vasconcelos, R. O. (2021). Impact of noise on development, physiological stress and behavioural patterns in larval zebrafish. *Sci. Rep.* **11**, 6615. doi:10.1038/s41598-021-85296-1
- Le Prell, C. G., Yamashita, D., Minami, S. B., Yamasoba, T. and Miller, J. M. (2007). Mechanisms of noise-induced hearing loss indicate multiple methods of prevention. *Hear. Res.* **226**, 22-43. doi:10.1016/j.heares.2006.10.006
- Leis, J. M. and Lockett, M. M. (2005). Localization of reef sounds by settlement-stage larvae of coral-reef fishes (Pomacentridae). *Bull. Mar. Sci.* **76**, 715-724.
- Lim, D. J. (1986). Effects of noise and ototoxic drugs at the cellular level in the cochlea: a review. *Am. J. Otolaryngol. Head Neck Med. Surg.* **7**, 73-99. doi:10.1016/S0196-0709(86)80037-0

- Lu, Z. and Desmidt, A. A.** (2013). Early development of hearing in zebrafish. *J. Assoc. Res. Otolaryngol.* **14**, 509-521. doi:10.1007/s10162-013-0386-z
- Mancera, K. F., Lisle, A., Allavena, R. and Phillips, C. J. C.** (2017). The effects of mining machinery noise of different frequencies on the behaviour, faecal corticosterone and tissue morphology of wild mice (*Mus musculus*). *Appl. Anim. Behav. Sci.* **197**, 81-89. doi:10.1016/j.applanim.2017.08.008
- Monroe, J. D., Rajadinakaran, G. and Smith, M. E.** (2015). Sensory hair cell death and regeneration in fishes. *Front. Cell. Neurosci.* **9**, 1-18. doi:10.3389/fncel.2015.00131
- Monroe, J. D., Manning, D. P., Uribe, P. M., Bhandiwad, A., Sisneros, J. A., Smith, M. E. and Coffin, A. B.** (2016). Hearing sensitivity differs between zebrafish lines used in auditory research. *Hear. Res.* **341**, 220-231. doi:10.1016/j.heares.2016.09.004
- Montgomery, J. C., Jeffs, A., Simpson, S. D., Meekan, M. and Tindle, C.** (2006). Sound as an orientation cue for the pelagic larvae of reef fishes and decapod crustaceans. *Adv. Mar. Biol.* **51**, 143-196. doi:10.1016/S0065-2881(06)51003-X
- Mueller, C. A.** (2018). Critical windows in animal development: interactions between environment, phenotype, and time. In *Development and Environment*, pp. 41-72. Springer. doi:10.1007/978-3-319-75935-7_3
- Nedelec, S. L., Campbell, J., Radford, A. N., Simpson, S. D. and Merchant, N. D.** (2016). Particle motion: the missing link in underwater acoustic ecology. *Methods Ecol. Evol.* **7**, 836-842. doi:10.1111/2041-210X.12544
- Ortega, C. P., Berten, L., Rigo, P., Aubrun, F., Nedelec, S. L., Simpson, S. D. and Lecchini, D.** (2012). Effects of noise pollution on birds: a brief review of our knowledge. *Source: Ornithological Monographs Ornithological Monographs* **74**, 6-22. doi:10.1525/om.2012.74.1.6.6
- Parmentier, E., Berten, L., Rigo, P., Aubrun, F., Nedelec, S. L., Simpson, S. D. and Lecchini, D.** (2015). The influence of various reef sounds on coral-fish larvae behaviour. *J. Fish Biol.* **86**, 1507-1518. doi:10.1111/jfb.12651
- Peris, E.** (2020). Environmental noise in Europe - 2020. *Eur. Environ. Agency* **1**, 104.
- Perry, C.** (1998). *A review of the impact of anthropogenic noise on cetaceans*. Report SC/50/E9 to the Scientific Committee of the International Whaling Commission, 1-27.
- Popper, A. N. and Fay, R. R.** (1973). Sound detection and processing by teleost fishes: a critical review. *J. Acoust. Soc. Am.* **53**, 1515-1529. doi:10.1121/1.1913496
- Popper, A. N. and Fay, R. R.** (1993). Sound detection and processing by fish: critical review and major research questions. *Brain Behav. Evol.* **41**, 14-38. doi:10.1159/000113821
- Popper, A. N., Smith, M. E., Cott, P. A., Hanna, B. W., Macgillivray, A. O., Austin, M. E. and Mann, D. A.** (2005). Effects of exposure to seismic airgun use on hearing of three fish species. *J. Acoust. Soc. Am.* **117**, 3958-3971. doi:10.1121/1.1904386
- Purser, J. and Radford, A. N.** (2011). Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks (*Gasterosteus aculeatus*). *PLoS ONE* **6**, e17478. doi:10.1371/journal.pone.0017478
- Rabin, L. A., Coss, R. G. and Owings, D. H.** (2006). The effects of wind turbines on antipredator behavior in California ground squirrels (*Spermophilus beecheyi*). *Biol. Conserv.* **131**, 410-420. doi:10.1016/j.biocon.2006.02.016
- Rohmann, K. N., Tripp, J. A., Genova, R. M. and Bass, A. H.** (2014). Manipulation of BK channel expression is sufficient to alter auditory hair cell thresholds in larval zebrafish. *J. Exp. Biol.* **217**, 2531-2539. doi:10.1242/jeb.103093
- Rohmann, K. N. and Bass, A. H.** (2011). Seasonal plasticity of auditory hair cell frequency sensitivity correlates with plasma steroid levels in vocal fish. *J. Exp. Biol.* **214**, 1931-1942. doi:10.1242/jeb.054114
- Scholik, A. R. and Yan, H. Y.** (2001). Effects of underwater noise on auditory sensitivity of a cyprinid fish. *Hear. Res.* **152**, 17-24. doi:10.1016/S0378-5955(00)00213-6
- Schuck, J. B. and Smith, M. E.** (2009). Cell proliferation follows acoustically-induced hair cell bundle loss in the zebrafish sacculus. *Hear. Res.* **253**, 67-76. doi:10.1016/j.heares.2009.03.008
- Simmons, A. M. and Narins, P. M.** (2018). Effects of anthropogenic noise on amphibians and reptiles. *Springer Handbook of Auditory Research* **66**, 179-208. doi:10.1007/978-1-4939-8574-6_7
- Simpson, S. D., Meekan, M. G., Mccauley, R. D. and Jeffs, A.** (2004). Attraction of settlement-stage coral reef fishes to reef noise. *Mar. Ecol. Prog. Ser.* **276**, 263-268. doi:10.3354/meps276263
- Simpson, S. D., Yan, H. Y., Wittenrich, M. L. and Meekan, M. G.** (2005). Response of embryonic coral reef fishes (Pomacentridae: Amphiprion spp.) to noise. *Mar. Ecol. Prog. Ser.* **287**, 201-208. doi:10.3354/meps287201
- Sisneros, J. A.** (2009). Seasonal plasticity of auditory sacculus sensitivity in the vocal plainfin midshipman fish, *Porichthys notatus*. *J. Neurophysiol.* **102**, 1121-1131. doi:10.1152/jn.00236.2009
- Smith, M. E., Kane, A. S. and Popper, A. N.** (2004). Acoustical stress and hearing sensitivity in fishes: Does the linear threshold shift hypothesis hold water? *J. Exp. Biol.* **207**, 3591-3602. doi:10.1242/jeb.01188
- Smith, M. E., Coffin, A. B., Miller, D. L. and Popper, A. N.** (2006). Anatomical and functional recovery of the goldfish (*Carassius auratus*) ear following noise exposure. *J. Exp. Biol.* **209**, 4193-4202. doi:10.1242/jeb.02490
- Stawicki, T. M., Esterberg, R., Hailey, D. W., Raible, D. W. and Rubel, E. W.** (2015). Using the zebrafish lateral line to uncover novel mechanisms of action and prevention in drug-induced hair cell death. *Front. Cell. Neurosci.* **9**, 1-7. doi:10.3389/fncel.2015.00046
- Strykowski, J. L. and Schech, J. M.** (2015). Effectiveness of recommended euthanasia methods in larval zebrafish (*Danio rerio*). *J. Am. Assoc. Lab. Anim. Sci.* **54**, 81-84.
- Tabor, K. M., Bergeron, S. A., Horstick, E. J., Jordan, D. C., Aho, V., Porkka-Heiskanen, T., Haspel, G. and Burgess, H. A.** (2014). Direct activation of the Mauthner cell by electric field pulses drives ultrarapid escape responses. *J. Neurophysiol.* **112**, 834-844. doi:10.1152/jn.00228.2014
- Tanimoto, M., Ota, Y., Inoue, M. and Oda, Y.** (2011). Origin of inner ear hair cells: Morphological and functional differentiation from ciliary cells into hair cells in zebrafish inner ear. *J. Neurosci.* **31**, 3784-3794. doi:10.1523/JNEUROSCI.5554-10.2011
- Ton, C. and Parg, C.** (2005). The use of zebrafish for assessing ototoxic and otoprotective agents. *Hear. Res.* **208**, 79-88. doi:10.1016/j.heares.2005.05.005
- Uribe, P. M., Villapando, B. K., Lawton, K. J., Fang, Z., Gritsenko, D., Bhandiwad, A., Sisneros, J. A., Xu, J. and Coffin, A. B.** (2018). Larval zebrafish lateral line as a model for acoustic trauma. *ENEURO* **5**, ENEURO.0206-18. doi:10.1523/ENEURO.0206-18.2018
- Valero, M. D., Burton, J. A., Hauser, S. N., Hackett, T. A., Ramachandran, R. and Liberman, M. C.** (2017). Noise-induced cochlear synaptopathy in rhesus monkeys (*Macaca mulatta*). *Hear. Res.* **353**, 213-223. doi:10.1016/j.heares.2017.07.003
- Vanwallieghem, G., Heap, L. A. and Scott, E. K.** (2017). A profile of auditory-responsive neurons in the larval zebrafish brain. *J. Comp. Neurol.* **525**, 3031-3043. doi:10.1002/cne.24258
- Vasconcelos, R. O., Sisneros, J. A., Amorim, M. C. P. and Fonseca, P. J.** (2011). Auditory sacculus sensitivity of the vocal Lusitanian toadfish: low frequency tuning allows acoustic communication throughout the year. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **197**, 903-913. doi:10.1007/s00359-011-0651-8
- Vasconcelos, R. O., Alderks, P. W., Ramos, A., Fonseca, P. J., Amorim, M. C. P. and Sisneros, J. A.** (2015). Vocal differentiation parallels development of auditory sacculus sensitivity in a highly soniferous fish. *J. Exp. Biol.* **218**, 2864-2872. doi:10.1242/jeb.123059
- Vermeij, M. J. A., Marhaver, K. L., Huijbers, C. M., Nagelkerken, I. and Simpson, S. D.** (2010). Coral larvae move toward reef sounds. *PLoS ONE* **5**, 3-6. doi:10.1371/journal.pone.0010660
- Wang, Y., Hirose, K. and Liberman, M. C.** (2002). Dynamics of noise-induced cellular injury and repair in the mouse cochlea. *J. Assoc. Res. Otolaryngol.* **3**, 248-268. doi:10.1007/s101620020028
- Wang, C., Zhong, Z., Sun, P., Zhong, H., Li, H. and Chen, F.** (2017). Evaluation of the hair cell regeneration in zebrafish larvae by measuring and quantifying the startle responses. *Neural Plast.* **2017**, 1-8. doi:10.1155/2017/8283075
- Weiss, S. A., Preuss, T. and Faber, D. S.** (2008). A role of electrical inhibition in sensorimotor integration. *Proc. Natl. Acad. Sci. USA* **105**, 18047-18052. doi:10.1073/pnas.0806145105
- Wong, A. C. Y. and Ryan, A. F.** (2015). Mechanisms of sensorineural cell damage, death and survival in the cochlea. *Front. Aging Neurosci.* **7**, 1-15. doi:10.3389/fnagi.2015.00058
- World Health Organization** (2015). Hearing loss due to recreational exposure to loud sounds: a review. *World Health Organization*. <https://apps.who.int/iris/handle/10665/154589>
- Yang, C.-H., Schrepfer, T. and Schacht, J.** (2015). Age-related hearing impairment and the triad of acquired hearing loss. *Front. Cell. Neurosci.* **9**, 1-12. doi:10.3389/fncel.2015.000276
- Yao, Q., Desmidt, A. A., Tekin, M., Liu, X. and Lu, Z.** (2016). Hearing assessment in zebrafish during the first week postfertilization. *Zebrafish* **13**, 79-86. doi:10.1089/zeb.2015.1166
- Zheng, F. and Zuo, J.** (2017). Cochlear hair cell regeneration after noise-induced hearing loss: does regeneration follow development? *Hear. Res.* **349**, 182-196. doi:10.1016/j.heares.2016.12.011