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Characterization of the Natural Soundscape of Zebrafish and Comparison with the Captive Noise Conditions

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Abstract

Zebrafish is a well-established model organism in hearing research. Although the acoustic environment is known to shape the structure and sensitivity of auditory systems, there is no information on the natural soundscape of this species. Moreover, zebrafish are typically reared in large-scale housing systems (HS), although their acoustic properties and potential effects on hearing remain unknown. We characterized the soundscape of both zebrafish natural habitats and laboratory captive conditions, and discussed possible impact on auditory sensitivity. Sound recordings were conducted in five distinct zebrafish habitats (Southwest India), from quieter stagnant environments with diverse biological/abiotic sounds to louder watercourses characterized by current and moving substrate sounds. Sound pressure level (SPL) varied between 98 and 126 dB re 1 μPa. Sound spectra presented most energy below 3000 Hz and quieter noise windows were found in the noisiest habitats matching the species best hearing range. Contrastingly, recordings from three zebrafish HS revealed higher SPL (122–143 dB) and most energy below 1000 Hz with more spectral peaks, which might cause significant auditory masking. This study establishes an important ground for future research on the adaptation of zebrafish auditory system to the natural soundscapes, and highlights the importance of controlling noise conditions in captivity.

Keywords: ambient noise, soundscape, natural habitat, hearing sensitivity, sound pressure level

Introduction

In aquatic environments, sound acts as an efficient information carrier for fish, which have evolved a remarkable diversity of auditory structures to enhance their hearing sense.[1] By listening to the aquatic background noise, fish can extract critical biotic information about the presence of conspecifics and heterospecifics, including potential mates, prey, and predators.[2,3] Moreover, they can also perceive important abiotic information for orientation, such as sounds derived from wind, water current, cavitation, and moving substrate.[2,4] Since fish species can detect and process both sound pressure and particle motion, perform sound source segregation and auditory scene analysis, the underwater soundscapes can be extremely complex in information, and even richer compared with terrestrial acoustic environments.[5,6]

It is known that underwater soundscapes play an important role shaping auditory structures and sensitivity of fish.[7] Several studies indicate that species are often well adapted to the lowest noise levels encountered in their natural habitats.[7–9] When background noise levels are elevated due to anthropogenic noise sources, fish may experience physiological stress and auditory impairment, namely masking, temporary hearing loss, and damage of the sensory auditory hair cells.[10–12]

Elevated background noise is commonly present in fish aquaculture systems. Large-scale housing systems (HS) often use equipment, such as air and water pumps, filtration systems, harvesters, feeding and maintenance machinery, which produce noise especially below 1000 Hz.[13–15] Consequently, fish species are chronically exposed to elevated noise that is usually within their sensitive hearing ranges. Only very few studies investigated the effects of background noise from HS on fish and results showed reduced egg viability and growth rates,[16,17] but also no developmental and physiological stress effects.[15,18] Information on the effects of captive noise conditions on fish hearing is extremely limited. Wysocki *et al.*[15] did not find an impact of increasing tank noise levels from 115 to 150 dB re 1 μPa root mean squared (RMS) on the hearing thresholds in the rainbow trout, *Oncorhynchus mykiss* (Salmonidae). However, Gutscher *et al.*[19] investigated the effect of aquarium noise with different filtering systems on the hearing in the goldfish *Carassius auratus* (Cyprinidae), an

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ostariophysan species with accessory hearing structures, namely, Weberian ossicles that link the swim bladder to the inner ear increasing auditory sensitivity and frequency range detection. The authors found considerable auditory threshold shifts (masking) by all noise types (threshold shifts of 15–19 dB) within the species best hearing range (600–1000 Hz). Therefore, it is likely that some of the published studies concerning behavioral and physiological response of fish, including hearing sensitivity, are affected by the elevated noise conditions in the laboratory housing facilities. This might be a particular issue for ostariophysan species with accessory hearing structures and enhanced auditory sensitivity.

The zebrafish, *Danio rerio* (Cyprinidae), has become an important model organism to investigate the molecular basis of inner ear development and function, human deafness, and hair cell regeneration.[20–22] This species has a typical vertebrate inner ear at the cellular level and its anatomy and development have been intensively described.[21–23] The zebrafish is an ostariophysan species with Weberian ossicles linking the swim bladder to the inner ear[24] with best hearing range between 600 and 1000 Hz.[22,25] Even though zebrafish has become a well-established model for hearing research, there is no information available on the soundscapes of its natural environment. This species is found in the North Eastern and South Western India, Nepal, Bangladesh, South Pakistan, and Northern Myanmar and inhabits diverse freshwater habitats, ranging from stagnant waters ponds to main river courses,[26] which may have shaped its auditory structures and hearing abilities.[7]

Moreover, zebrafish are commonly maintained in large-scale laboratory facilities while being used for research.[27] Such environments are characterized by elevated noise levels, probably resulting from aerators, air and water pumps, water circulation, and feeding machinery.[28] The noise levels and spectral features of typical zebrafish HS, how they compare to the natural habitat conditions, and their potential to affect species' hearing abilities have never been investigated.

The present study aimed to (1) characterize the variability of soundscapes of typical zebrafish freshwater habitats in Southwest India, from slow-flow backwaters/ponds to main river courses; (2) investigate the noise conditions of typical zebrafish housing facilities; and (3) compare the species auditory sensitivity with the spectral features of both natural and artificial noise environments.

Methods

Sound recordings in the natural habitats

The study area was selected based on previously reported distribution of zebrafish in Karnataka, Southwest India.[29] Among the different possible locations, we selected a variety of habitats with different hydrological traits to characterize the diversity in soundscapes (Table 1). The criteria to choose the recording locations were: selection of a site that would be representative of that specific habitat (pool, backwaters, main watercourse); identification of zebrafish *D. rerio* shoals; and accessibility with the recording equipment. We also selected recording locations where prior studies[29] were conducted and further details on ecological features can be found.

In all recording locations we confirmed the occurrence of zebrafish by observation and capture using rectangular hand nets and fine mesh seines (mesh grid size varying between 1 and 3 mm), in collaboration with M. Arunachalam (Manonmaniam Sundaranar University, India). All sound recordings were performed under tropical dry season conditions in the absence or with weak wind (<4 km/h) and no rain. Comparing acoustic conditions between dry and wet seasons would be relevant and should be considered in future research.

The zebrafish were mostly found in shallow water masses of low flow with sand, lime, silt, and/or bedrock substrate; in small secondary or tertiary channels of a main river; or in adjacent backwaters, but also along the margins of a main river. The species behavior varied from stationary swimming compact shoals countering the water flow in the Tunga River of Shringeri (SH) to free swimming loose shoals in the riverbed pools of Kallahalli (KA) (Table 1). Ambient noise recordings and sound pressure level (SPL) measurements were conducted in five distinct locations (Fig. 1, Table 1): (1) AC, Achacanni, west flowing secondary stream between waterfalls (circa 50 m away from nearest waterfall), tributary of Sharavati river near Sharavati natural reserve; (2) KA, Kallahalli, natural water pool carved in the riverbed of the southeast-flowing Kaveri river; (3) SIS, Sidi Halla with sandy substrate, tertiary southwest-flowing stream adjacent to paddy riversides; (4) SIR, Sidi Halla with rocky substrate, secondary southwest-flowing channel in the same basin as SIS; and (5) SH, Shringeri (west-flowing Tunga River), main river course with faster water flow.

Ambient noise was recorded at a sampling rate of 44.1 kHz using a hydrophone (Aquarian Audio H2a-XLR-15, Anacortes, WA; frequency range: 10–100 kHz ±4 dB; voltage sensitivity: -180 dB re 1 V/μPa^{-1}) connected to an A/D converter phantom-powered device (Edirol UA-25; Roland, Tokyo, Japan) and then to a laptop computer running Raven Pro 64 1.5 software (The Cornell Lab of Ornithology, Ithaca, NY). SPLs were measured with a hydrophone (Brüel & Kjær 8101, Naerum, Denmark; frequency range: 1–80 kHz ±2 dB; voltage sensitivity: -184 dB re 1 V/μPa^{-1}) connected to a hand-held sound level meter (Brüel & Kjær 2250). The hydrophones were attached to a pole and positioned at about 15–20 cm depth, avoiding direct contact with substrate and vegetation. The hydrophones were positioned within the same location (<1 m), where the zebrafish were observed.

Ambient noise recordings and SPL measurements followed previously described protocols.[30,31] Sound recordings were 15 min each and two consecutive recordings were conducted per site. The equivalent continuous SPL (L_{Zeq}; flat weighting: 6.3–20 kHz) averaged over 60 s was obtained six times per site, that is, three times immediately before and after sound recording session. L_{Zeq} (also known as L_{Leq}) is a measure of averaged energy in a varying sound field and is commonly used in environmental noise studies (ISO 1996 2003).

We considered just one sampling site per location, except in Sidi Halla (SIS and SIR), which could underestimate the potential variation within each location. However, we preferred to characterize a single sampling site per habitat, where zebrafish were observed by conducting sound recordings for relatively long periods of time than usually reported, and consider a representative range of different zebrafish habitats.

Table 1. Natural Habitat Characterization at Sampling Sites

Recording locations[a]	*GPS coordinates*	*Habitat*[b]	*Elevation (m)*	*Water temp. (°C)*	*Depth (cm)*	*Flow (cm/s)*	*Water visibility*	*Substrate*	*Main noise sources*	*Zebrafish occurrence (no. of individuals)*
KA Kallahalli	12.3112° N/76.2160° E	Shallow pool connected to main riverbed	599	27.5–28	20–50	5.5–6.2	Turbid, bottom not visible	Bedrock, lime, silt	Cavitation, insects	Free-swimming loose shoals inside well (5–10)
AC Achacanni	13.4848° N/75.1034° E	Shallow and narrow second-order stream	615	19.5–20	10–25	5.2–5.8	Clear, bottom visible	Sand, leaves, litter, lime, silt	Cavitation, moving substrate, insects, birds	Stationary compact shoals countering current (15–25)
SIS Sidi Halla (sandy)	13.3945° N/75.1822° E	Third-order shallow stream	772	22–23	10–30	—	Turbid, bottom not visible	Sand, lime, silt	Moving substrate, birds, insects	Free-swimming compact shoals (5–10)
SIR Sidi Halla (rocky)	13.3955° N/75.1251° E	Third-order narrow stream	771	21–22	20–35	—	Clear, bottom visible	Bedrock, gravel, sand	Water current/ cavitation, moving substrate, insects	Stationary compact shoal countering current (5–10)
SH Shringeri	13.5521° N/75.2642° E	Main river stream	680	22–22.5	35–50	6.3–6.8	Clear, bottom visible	Bedrock, gravel, boulders, sand	Water current/ cavitation, moving gravel, and boulders	Compact shoal behind substrate bulk (5–10)

[a]Recording locations are presented in Arunachalam *et al.*,[29] where further details on habitat description during dry season can be found.
[b]Vegetation cover was present as dense (AC) and sparse (SIS) hanging canopy, and riparian riverside plants (AC, SIS, SIR), whereas it was absent at SH and KA.
—, designates missing values for SIS and SIR.

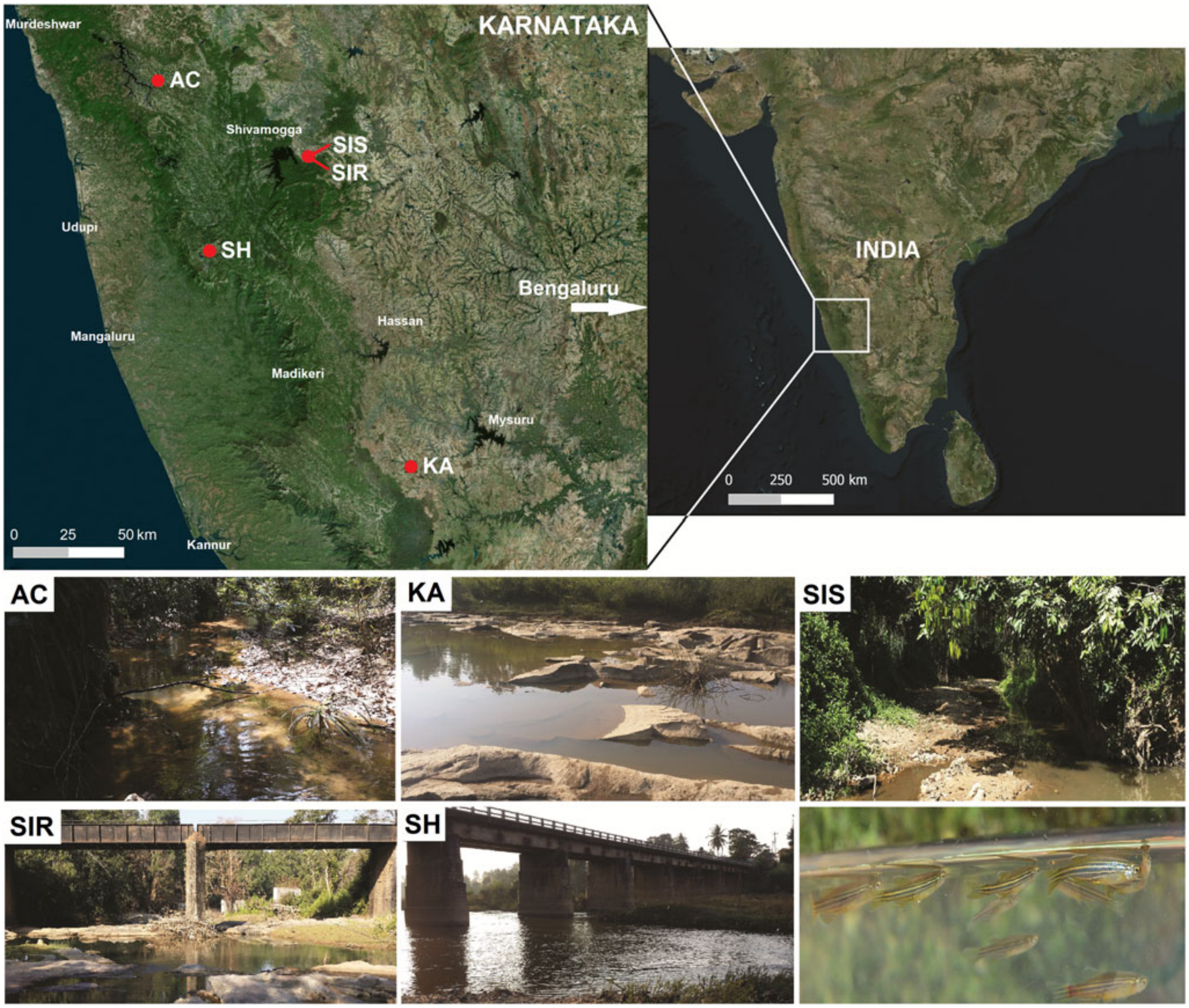

FIG. 1. Map of India (*top*) showing the geographical location of the different zebrafish natural habitats selected for this study in Southwest India (Karnataka state): AC—Achacanni, shallow low-flow stream near Hosanagara at the border of Sharavati Valley Wildlife Sanctuary; KA—Kallahalli, natural wells connected to Kaveri river; SIS—Sidi Halla (sandy), low-flow stream with sandy substrate near Shivamogga; SIR—Sidi Halla (rocky), medium-flow stream with rocky substrate adjacent to SIS and SH—Shringeri, faster flow main stream of Tunga River. In all study locations, the team confirmed the occurence of zebrafish (*Danio rerio*) (*bottom right*, specimens captured at SIR). Color images available online at www.liebertpub.com/zeb

Sound recordings in laboratory housing systems

We selected three typical zebrafish HS from different laboratory facilities in Macau, namely at the University of Saint Joseph and the University of Macau. The selected HS were: (1) HS1, stand-alone system with five double-sided shelves and frame-integrated filtering and pumping system equipped with 224 acrylic tanks (1–10 L), model AAB-074-AA-A, Yakos 65, Taiwan; (2) HS2, stand-alone system with similar configuration to HS1 equipped with 168 acrylic tanks (1–10 L), model AAB-100-AA-A, Yakos 65, Taiwan; and (3) HS3, multilinking system with external water treatment unit connecting three single-sided rack frames equipped with 65 acrylic tanks (1–8 L) with pumps and filters configured in an external unit connected to an automatic feeder (Triton; ZebTEC, Techniplast, Italy) (Fig. 2).

Sound recordings and SPL measurements followed the same abovementioned protocol, with the exception that the hydrophones were placed in the middle of the fish tanks and submerged at 5 cm from the bottom. Three recording points were selected in each housing system to better characterize the noise variability attending to their distance to the main sound source, the water pump, and filtering system (Fig. 2). SPL measurements were done in following locations for all HS: 8–10 L tank in the bottom (30–40 cm distance to water pump/filters); 3.5 L tank in the middle (140–150 cm distance), and 1 L tank at the top (240–250 cm distance). These locations varied in SPL but presented similar spectral composition (sound energy distribution across frequencies). Only the middle recording location was considered while comparing SPL across different HS and relative to field noise levels. Considering more than one HS location (at different

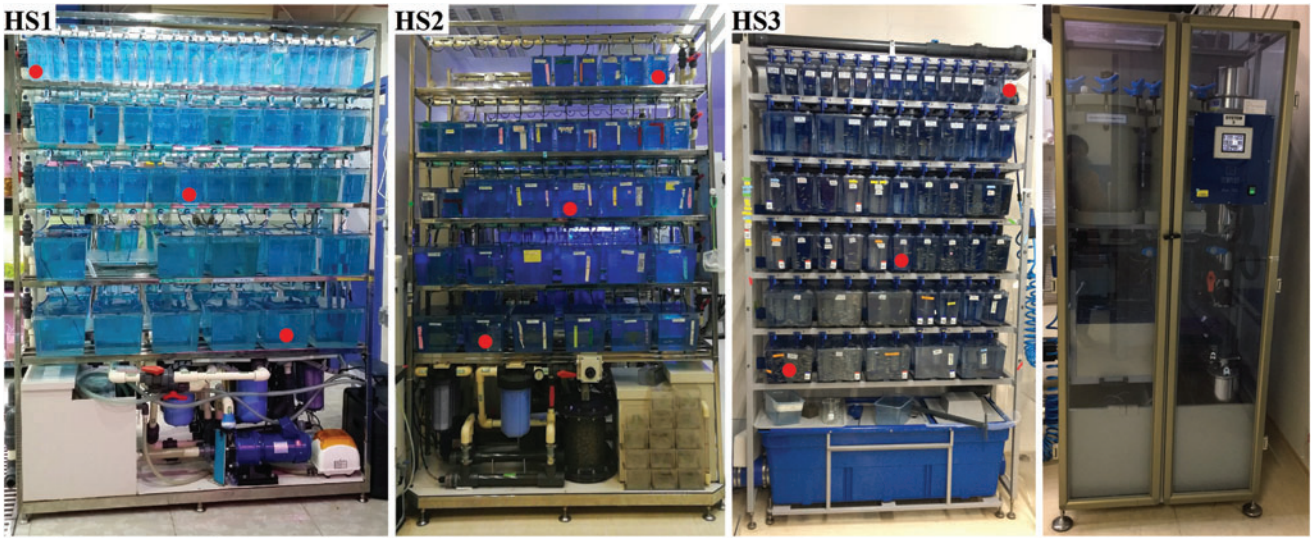

FIG. 2. Three representative zebrafish housing systems (HS) considered in this study to characterize noise conditions in captivity. *Red dots* indicate the fish tanks selected for the recordings of SPL measurements. HS1 and HS2—stand-alone systems with frame-integrated filtering and pumping system–models AAB-074-AA-A and AAB-100-AA-A, respectively (Yakos 65, Taiwan); HS3—multilinking system with external water treatment unit connecting three stand-alone racks with pumps and filters configured in an external compartment. (*right picture*) (ZebTEC, Techniplast, Italy). Color images available online at www.liebertpub.com/zeb

distances to main noise source) in such analysis would have increased the SPL variability, and this would not have been consistent with what zebrafish individuals experience when they are housed in a particular location.

Sound analysis

Sound analysis was performed using Adobe Audition 3.0 (Adobe Systems, Inc., San Jose, CA). Natural habitat sound files were first inspected regarding potential artifacts and presence of anthropogenic noise. Since the purpose of this study was to characterize the zebrafish natural soundscape, various sounds from human activities (e.g., traffic, bridge vibrations, and people talking) and other recording artifacts (hydrophone vibrating with current or touching substrate) were removed. Even though anthropogenic sounds were occasionally part of the soundscape in several locations, studying such noise sources was not the scope of the present study. A final sound file of 10–15 min was created for each habitat, providing a representative characterization of the variability of the natural soundscape with occasional low-amplitude anthropogenic noise.

The relative fast Fourier transformation (FFT) of the final sound recordings representing each location was calculated (16,384 and 2048 FFT size, overlap 50%, Blackman–Harris window). Both power spectral density (PSD) level (given in dB re 1 μ Pa^2/Hz) and absolute sound spectra level (dB re 1 μ Pa) were determined using the averaged L_{Zeq} value calculated per site and following previously described procedures.[30,32] The PSD level was further calculated based on the equation (linearization): $Ai = 10^{(ai/10)}$, where Ai equals the linear spectral amplitude and ai is the logarithmic spectral amplitude. The values were then converted to PSD levels through the equation: PSD level $(\mathrm{dB}) = 10 \times \log_{10}\left(\frac{\sqrt{Ai}}{BW}\right)^2$, where BW represents bandwidth (spectral resolution).

Statistical analysis

Noise levels (L_{Zeq}) were compared between different natural habitats with Kruskal–Wallis H tests followed by Dunn's pairwise *post hoc* tests to verify habitat specific differences. Comparison of noise levels between artificial HS was performed with one-way ANOVA, followed by *post hoc* Tukey tests. Overall natural and artificial noise levels were compared with a Student's *t*-test. Parametric tests were used only when data were normally distributed and variances were homogeneous. The statistical analysis was performed with IBM SPSS v.22 (IBM Corp., USA).

Results

Characterization of the zebrafish natural soundscapes

The zebrafish occurred in a wide range of natural acoustic environments that differed significantly in the soundscape composition, SPL, and spectral features (see sound files in Supplementary Data; Supplementary Data are available online at www.liebertpub.com/zeb). The habitats varied from relatively quiet locations, such as slow-moving streams and riverside pool sites characterized by occasional sounds from water cavitation, moving substrate, and diverse biological activity, namely Sidi Halla (SIS), Achacanni (AC), and Kallahali (KA), to noisier environments like a main river exhibiting continuous water current and moving substrate sounds (SH) (Tables 1 and 2).

The biological sounds detected were mostly high pitched and produced by insects (main energy >2000 Hz) and birds (1000–7000 Hz) in the vicinity, whereas the abiotic sources consisted of water flowing and cavitation (700–4000 Hz) and moving substrate (900–5000 Hz). All these different sounds consisted of discrete events that occurred several times throughout the recordings, except for the water current sounds in SH that were continuously present (Fig. 3).

Table 2. Noise Levels (L_{ZEQ}) Determined in Five Zebrafish Natural Habitats (Karnataka, Southwest India) and in Three Typical Laboratory-Housing Systems

Environment	*Recording location*	*Mean ± SD*	*Min*	*Max*	*CV (%)*
Natural	SH	126.08 ± 0.30	125.49	126.19	0.23
	KA	105.83 ± 3.63	102.42	109.53	3.43
	AC	106.24 ± 0.96	104.74	107.40	0.91
	SIS	102.75 ± 0.32	102.34	103.12	0.32
	SIR	107.38 ± 3.50	104.24	110.83	3.26
Artificial	HS1				
	1	145.83 ± 0.08	145.70	145.90	0.06
	2	139.17 ± 0.33	138.80	139.50	0.24
	3	145.85 ± 0.46	145.20	146.40	0.32
	HS2				
	1	146.63 ± 0.43	146.20	147.00	0.29
	2	135.35 ± 0.80	133.80	136.10	0.59
	3	133.40 ± 0.35	133.10	133.90	0.26
	HS3				
	1	126.27 ± 0.98	125.20	127.60	0.78
	2	121.03 ± 0.19	120.80	121.30	0.15
	3	119.63 ± 0.08	119.50	119.70	0.07

Values are calculated from 4–6 averaged readings based on 60 s and are given in dB re 1 μPa. Natural habitats: SH—Shringeri, KA—Kallahalli, AC—Achacanni, SIS—Sidi Halla sandy, and SIR—Sidi Halla rocky. For each HS three recording points were considered at various distances to the main noise source (water pump and filtering system): (1) 30–40 cm, (2) 140–150 cm, and (3) 240–250 cm.
CV, coefficient of variation; HS, housing systems.

SPLs (or L_{Zeq}) varied between 102.75 ± 0.32 dB re 1 μPa (mean ± standard deviation) in a low-flow small stream (SIS) to 126.08 ± 0.30 dB in a main river course (SH) (Table 2, Fig. 4). Significant differences in SPL were found between the different recording sites (H (4, 27) = 19.05; $p < 0.001$). Pairwise *post hoc* comparisons revealed that Tunga River in Shringeri (SH) was significantly louder compared with all the other locations, as well as SIS in relation to SIR ($p < 0.05$).

The SPL variability within the same study site was the lowest at the noisiest habitat, the main river (SH). The difference between the minimum and maximum L_{Zeq} was 0.81 dB at SH (coefficient of variation or CV = 0.23%), whereas it was 7.11 dB at KA (CV = 3.43%). Within the same habitat type, namely the low-flow streams (KA, AC, SIS), the levels differed by up to 7.19 dB. In Sidi Halla, two recording locations were considered and the presence of faster water flow and different substrate in SIR (bedrock, gravel, and sand), compared with SIS (substrate sand, lime, and silt), probably contributed for the increase of circa 5 dB from 102.75 ± 0.32 to 107.38 ± 3.50 dB, respectively.

The spectral profiles varied considerably between natural habitats, although they all showed a general decline in energy toward higher frequencies (Fig. 3). The energy decline was more gradual in the shallow streams with lower water flow (AC, KA, and SIS), which presented most energy below 600–800 Hz. In the third-order stream SIR, besides the higher amplitude at low frequencies, an additional spectral peak was found at 2000–4000 Hz resulting from sounds mainly produced by nearby insects. In the main river (SH), more spectral energy was observed and a steep amplitude decline or ''noise window'' was detected within 100–2000 Hz.

Characterization of the ambient noise of zebrafish housing system

The two possible configurations of laboratory zebrafish HS were considered in this study, namely the ''stand-alone system'' with fish tanks, pump, and filters integrated in a single rack frame (HS1 and HS2), and a ''multilinking system'' with multiple racks containing fish tanks and a water deposit connected to an external enclosed module containing all pumps and filters (HS3).

The HS revealed SPLs ranging from 119.63 ± 0.08 dB in (HS3) to 146.63 ± 0.43 dB in (HS2) (Table 2, Figure 4; see sound files in Supplementary Data).

Significant differences in SPL were found in the middle tank of the different HS (F (2, 18) = 15,174; $p < 0.001$; $p < 0.0001$ *post-hoc* tests between all systems). The variability of SPLs for a specific location within each system was very low, namely of 0.20–2.40 dB (CV = 0.06–0.78%) for all the systems and recording points (Table 2, Figure 4). The SPLs were significantly dependent on the distance to the water pump and filters for two of the three systems (F (2, 54) = 7.95, $p < 0.05$). In both HS2 and HS3, fish were gradually exposed to higher noise levels with the proximity to these equipment. However, in HS1 the sound level did not follow the same gradual pattern and it was lower in the middle of the rack system (139.17 dB), compared with the closest and furthest recording points in relation to the pump/filters (145.83 and 145.85 dB, respectively).

The sound spectra from the different HS revealed most sound energy concentrated at low frequencies below 1000 Hz and a gradual decrease toward higher frequencies (Fig. 5). Several conspicuous energy peaks were observed specially in HS1 at 25, 45, 95, and 140 and between 180 and 1200 Hz. HS2 revealed peaks at 30, 50, 100, and 280 Hz, among others. Contrastingly, HS3 revealed comparatively a more gradual decline in energy distribution toward higher frequencies.

Natural versus artificial soundscapes: comparison with zebrafish hearing sensitivity

Comparison of mean SPLs between natural and artificial acoustic environments revealed overall significant differences

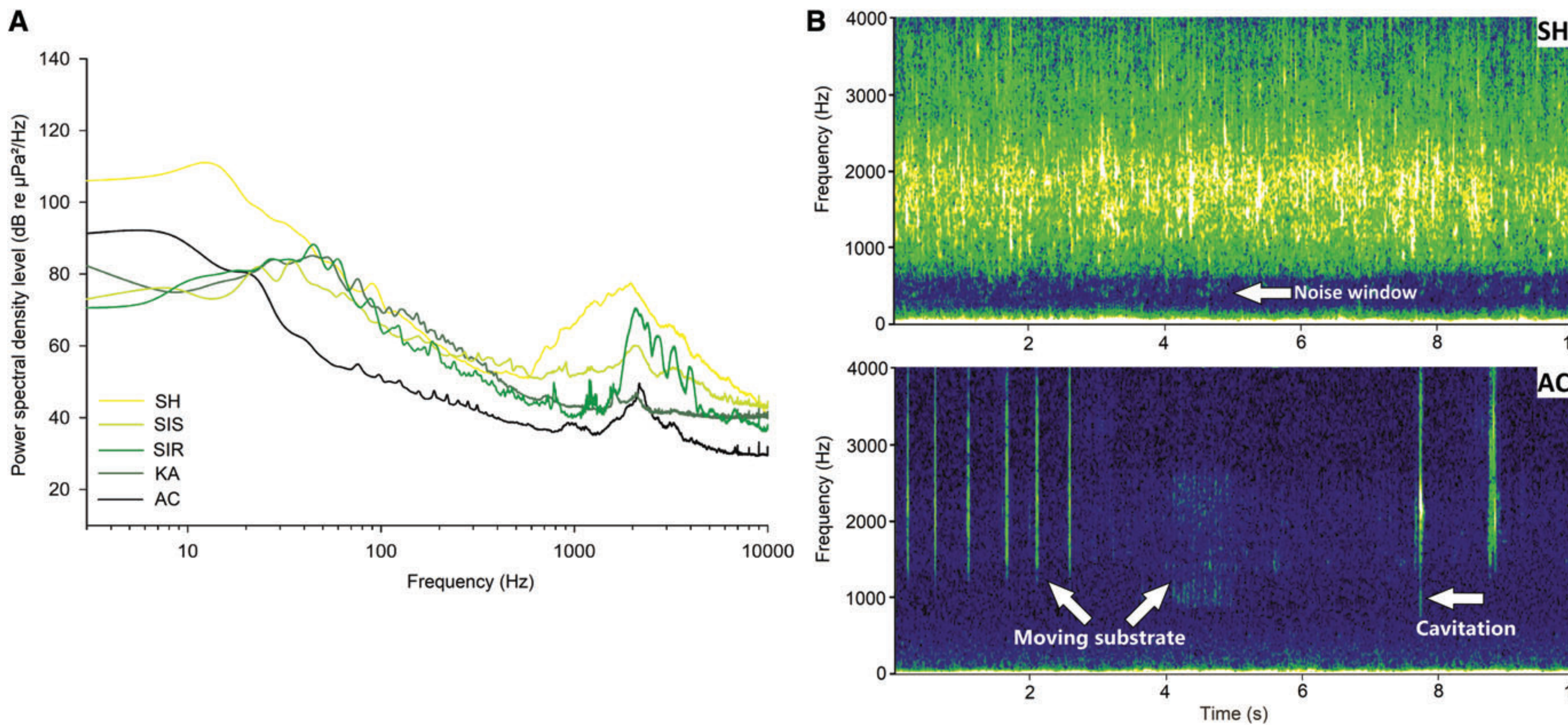

FIG. 3. (**A**) PSD of ambient noise from diverse zebrafish natural habitats from Southwest India (Karnataka). Sampling frequency: 44.1 kHz. FFT size 16,384. (**B**) Spectrograms of sound recordings from SH showing noise window and AC showing natural noise sources, FFT size 2048. SH, KA, AC, SIS, and SIR (for abbreviation refer to Fig. 1). FFT, fast Fourier transformation; PSD, power spectral density. Color images available online at www.liebertpub.com/zeb

($F (1, 43) = 78.88$, $p < 0.001$), with lower noise levels found in the natural habitats (Fig. 4). However, SPL variation was comparatively higher among natural environments compared with laboratory conditions (Table 2).

Comparing sound spectra of both types of soundscapes revealed noticeable differences (Fig. 6). While the shape of the spectral profiles from natural habitats showed most energy concentrated below 600–800 Hz and an energy peak in the noisiest habitats at 1000–4000 Hz due to diverse abiotic and biological sources, artificial HS presented most energy under 1000 Hz following a more irregular distribution pattern with multiple spectral peaks. Differences in sound amplitude between natural habitat and laboratory conditions were more noticeable below 1000 Hz with a variation of up to 60 dB.

Auditory sensitivity thresholds of wild-type zebrafish reported in previous studies[22,25,33] are quite variable with differences of up to 22 dB throughout the frequency detection range, with higher discrepancies at 100, 800, and 1500 Hz. Comparing both types of soundscape spectral profiles with the auditory sensitivity data, revealed a significant overlap between the sound energy of the artificial housing conditions and the species' hearing range (100–8000 Hz), especially for the stand-alone systems (HS1 and HS2). The spectral energy of these systems was up to 22.4 dB above the auditory thresholds. In contrast, the spectral profiles of most natural soundscapes were considerably below the zebrafish auditory thresholds. The fast-flowing river (SH), however, presented a conspicuous energy peak close to the lowest auditory thresholds within 800–2000 Hz. The best hearing range of the species (600–1000 Hz) matched a noise window within the soundscape of the noisiest habitat, but also a frequency range that exhibits the highest variability across all acoustic environments.

Discussion

To our knowledge, this is the first study investigating the acoustic properties of the natural freshwater habitats of zebrafish *D. rerio*, a widely used model organism in hearing research. Moreover, we provide an important comparison between the natural soundscapes with the artificial noise conditions found in zebrafish HS commonly used in research facilities. Our results showed significant higher noise levels

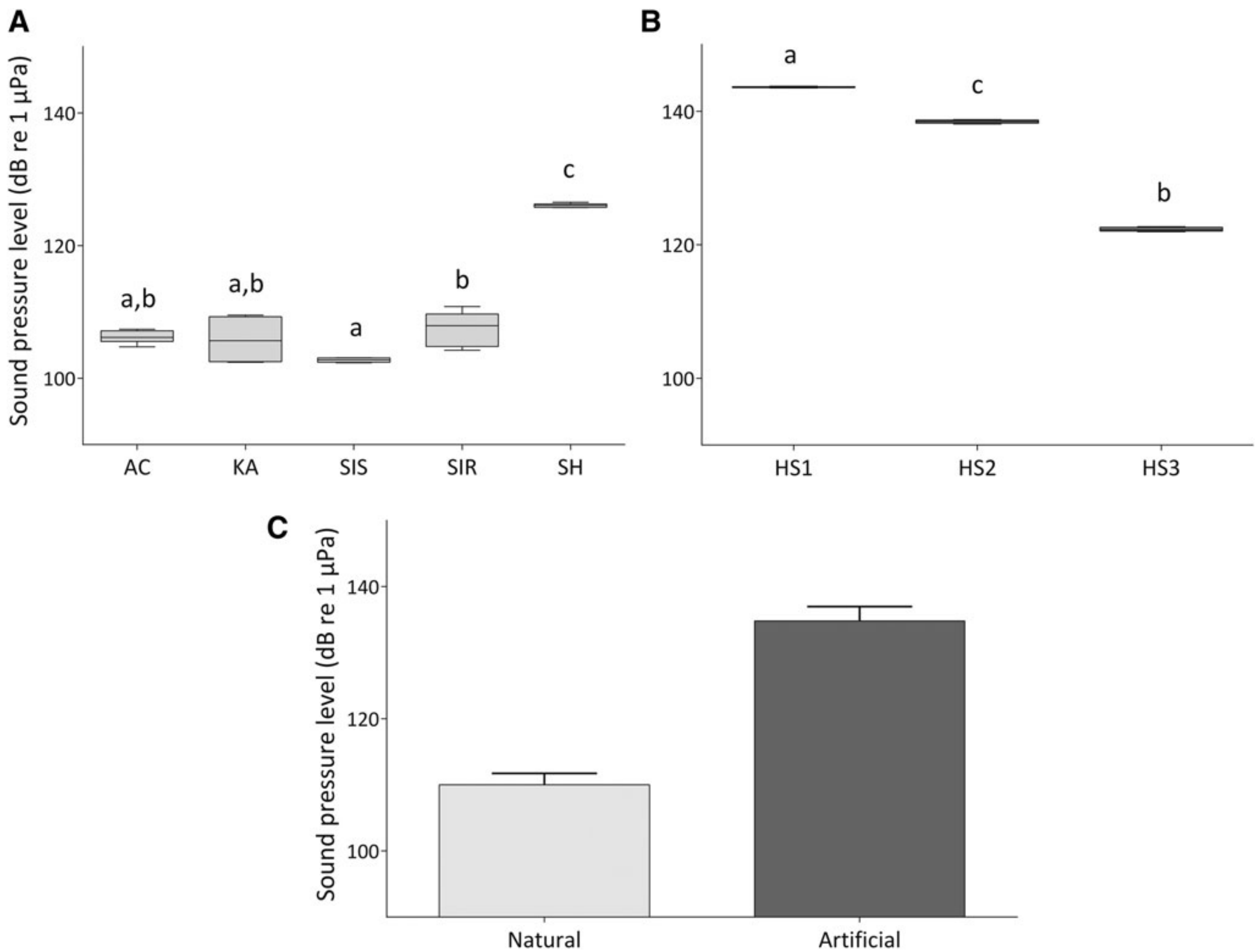

FIG. 4. Comparison of SPLs (linear equivalent, L_{Zeq}) between **(A)** zebrafish natural habitats ($H (4, 27) = 19.05$; $p < 0.001$), and **(B)** laboratory HS ($F (2, 18) = 15{,}174$; $p < 0.001$). Values are based on 60 s averaged measurements (L_{Zeq}), 4–6 per site. *Different letters* indicate statistically significant differences based on pairwise *post hoc* comparisons. Plots show medians and 10th, 25th, 75th, and 90th percentiles as *boxes* and *whiskers*. **(C)** Comparison between mean noise levels determined for natural habitats and artificial housing conditions ($F (1, 43) = 78.88$; $p < 0.001$). Plot shows mean and standard deviations.

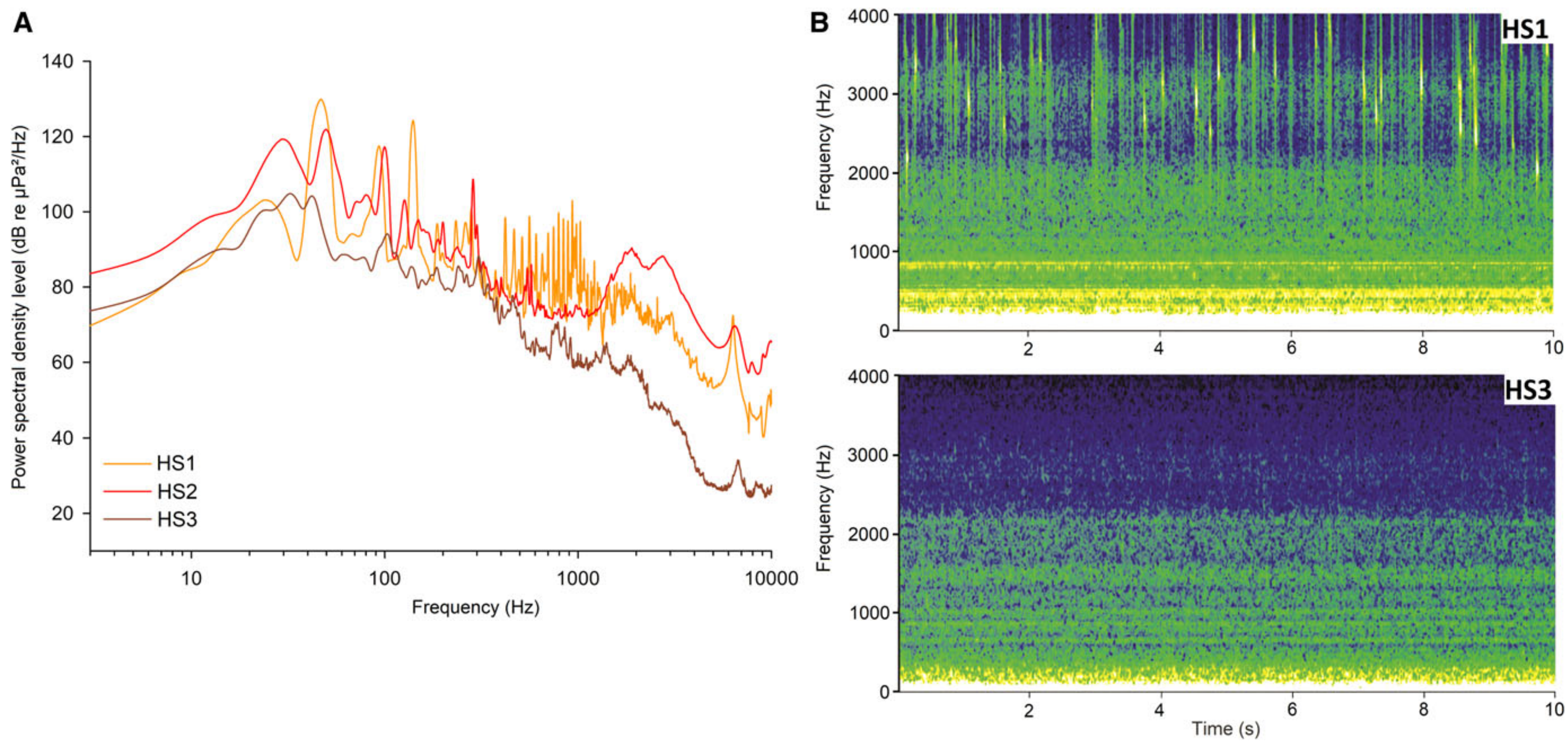

FIG. 5. (**A**) PSD of background noise from three typical zebrafish HS. Sampling frequency 44.1 kHz, FFT size 16,384. (**B**) Spectrograms of representative sound recordings from HS1 (stand-alone system, AAB-074-AA-A; Yakos 65, Taiwan) and HS3 (multilinking system with external pumping/filtering units; ZebTEC, Techniplast) with FFT size 2048. Color images available online at www.liebertpub.com/zeb

in HS compared with the natural environments, with potential to cause auditory masking. Additional differences were also found in sound spectral profiles and noise level variability.

Diversity in the soundscape of natural freshwater habitats

Over the past decades, there has been a growing interest on the variability of underwater soundscapes especially in marine ecosystems for commercial interests in fisheries but also for monitoring biodiversity for conservation purposes.[34–39] However, limited information is available on ambient noise from freshwater habitats.[7,8,30,31,40–42]

In freshwater habitats, the ambient noise levels are usually highly dependent on the water flow strength and substrate composition. Lakes and backwaters typically present lower noise levels compared with fast-flowing waters found in streams and rivers, with noise levels that can differ more than 40 dB.[7,30,31,41] In our study, the shallow water streams with low/medium flow and backwaters presented the lowest mean SPLs (circa 103–107 dB re 1 μPa). The sound sources were mostly not only abiotic from water current, cavitation, and moving substrate, but also biotic from calling insects and birds. Contrastingly, the main river course at Tunga River in Shringeri (SH) showed the highest SPL (126 dB re 1 μPa), most likely due to the higher water flow, larger water volume, and significant cavitation and transportation of sediment (sand, cobble, and boulders). The SPL values from quieter habitats were similar to the noise levels reported by Wysocki *et al.*[30] for backwaters (Gänsehaufen Traverse), pond (Prellenkirchen), and stream with bedrock substrate (Schwarza) in Austria, which corresponded to 99, 98, and 110 dB re 1 μPa, respectively. In the same study, the noise levels reported for a main river course and a stream were similar to the SPL recorded in the faster-flowing Tunga River. The Triesting stream, a typical Alpine creek with cobble and boulder substrate, revealed mean SPL of 124 dB re 1 μPa, and the free-flowing part of the Danube River revealed a noise level of about 135 dB.[30]

Other studies have reported ambient noise spectral profiles that indicate similar variability in noise levels of freshwater systems. For instance, Lugli and Fine[8] reported differences in spectral levels (1 Hz bandwidth) in several locations within two shallow stony streams in Italy (stream Stirone and river Serchio) with maximum SPL varying between 70 and 80 dB re 1 μPa (quiet locations) to 100–105 dB (rapids). Additionally, Crawford *et al.*[40] reported a noise background of about 75 dB re 1 μPa (RMS) at night in a shallow plain flood of a stream tributary of the Niger River (Mali). However, comparisons of noise levels across different studies are difficult since the mean SPL is not always described and spectral composition profiles are often given in different units and/or bandwidth.

Similar to previous studies, louder habitats, such as the Tunga River (SH), revealed lower variability in the noise levels compared with quieter environments.[30,31] Any additional noise in the soundscape in the quieter locations (including from anthropogenic sources and biological activity) contributed to a notable increase in the noise level.

Regarding noise spectral profiles, freshwater habitats such as rivers and streams typically present more energy at lower frequencies followed by a gradual noise level decline.[30,31,40] We also found a similar pattern of energy decline with increasing frequency in all zebrafish habitats investigated in this study. However, in the noisiest environment, Tunga River (SH), a noise window at lower frequencies was detected followed by a subsequent energy peak toward 2000 Hz. A low-frequency noise window has been reported in previous studies of freshwater habitats. Crawford *et al.*[40] reported a wider spectral window between 200 and 3000 Hz in the Niger River (Mali, Africa), followed by higher energy above 4 kHz. Lugli and Fine, and Lugli[8,41] identified noise windows at 100 Hz in a stony stream, as well as at 200–250 Hz in a vegetated spring and brackish lagoon. Wysocki *et al.*[30] reported lower spectral levels between 200 and 2000 Hz in a stream (Schawarza). The same authors found a similar pattern to the spectral composition of our noisiest study site (SH) in the Danube River (close to Danube island and free-flowing area), where a steep decline in spectral level was found around 200 Hz followed by a gradual increase toward 1000 Hz.

In summary, the soundscapes of zebrafish natural habitats investigated in this study revealed considerable diversity in sound levels and spectral composition, mostly resulting from differences in abiotic sources (volume and speed of water flow with cavitation and sediment composition and transportation). These differences might be important for zebrafish orientation and sound detection in the various acoustic environments.

Ambient noise in artificial housing systems

Very limited information is known on the acoustic properties of artificial tank systems and their impact on fish behavior, physiological stress, and hearing.[15,19,43,44] But it is known that vibrations and noise may cause stress and harm aquatic animals in laboratories (NRC 2011).[45] The studies available showed reduced fish egg viability and growth rates,[16,17] but also absence of developmental and physiological stress effects in the rainbow trout (*O. mykiss*), which do not have morphological hearing specializations.[15,18]

In our study, we investigated the noise levels and spectral features of three typical zebrafish HS, including stand-alone (frame built in filters and pump) and multilinking rack units (external WTU and pumps connected to racks). The SPL determined varied between 123 and 144 dB with significant higher noise levels in the stand-alone systems, indicating that great part of the background noise is caused by the proximity to the pumps/filters. Similar noise values were determined in other studies, although the information is scarce and difficult to compare due to distinct types of fish HS. For example, Gutscher *et al.*[19] found that an earthen pond (32 × 22 m, 1.8 m depth) without operating aerators presented spectral noise levels (L_{Leq}) below 100 dB re 1 μPa; whereas Wysocki *et al.*[15] reported SPL of about 149 dB re 1 μPaRMS in round fiberglass tanks (14 m diameter, 4 m depth) with recirculating system. Additionally, Bart *et al.*[14] compared the acoustic properties across a wide range of fish HS equipped with aeration systems and identified highest noise levels in larger fiberglass tanks (14 m diameter, 4 m depth) of about 153 dB re 1 μPa within 25–1000 Hz.

Moreover, the spectral composition of the ambient noise in the zebrafish HS investigated revealed most sound energy concentrated below 1000 Hz and a gradual decrease in SPL toward higher frequencies. Several energy peaks were

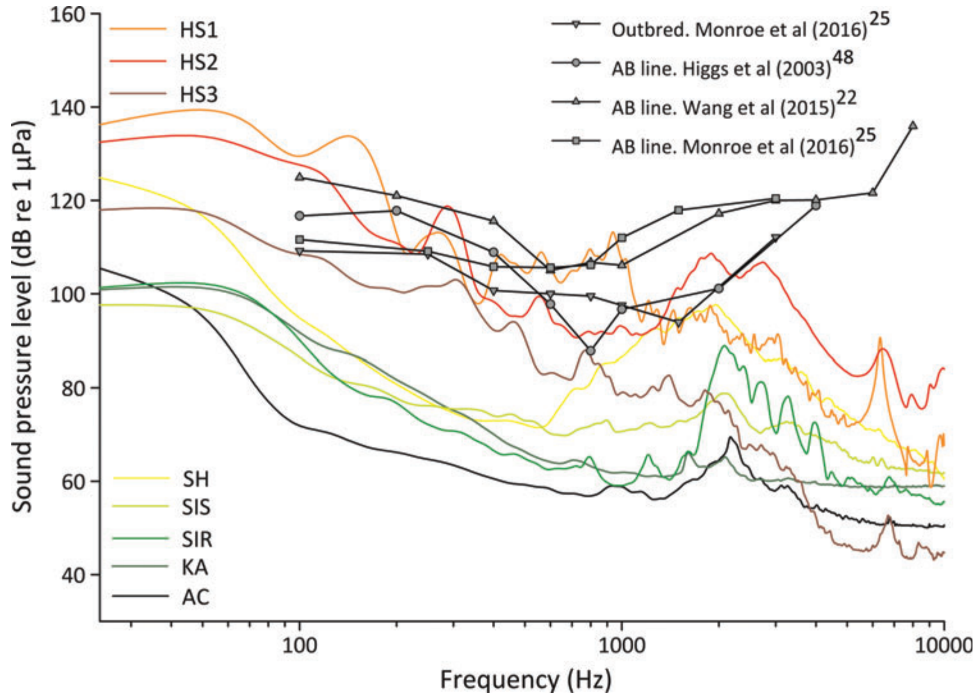

FIG. 6. Sound spectra from both natural and captive noise conditions compared with zebrafish audiograms (*gray bulleted lines*). Mean auditory thresholds indicated are from AB wild-type line[34,22,41] and wild-type line from Liles Tropical Fish, Inc. (Ruskin, FL).[34] KA—Kallahalli, natural wells connected to Kaveri river AC—Achacanni, shallow low- flow stream; SIS—Sidi Halla (sandy), low-flow stream; SIR—Sidi Halla (rocky), medium-flow stream; SH—Shringeri, fast-flow Tunga River; HS1 and HS2 (stand-alone systems from Yakos 65, Taiwan) and HS3 (multilinking system with external pumping/ filtering units from ZebTEC, Techniplast). Sampling frequency 44.1 kHz, FFT size 2048, Blackman Harris, 50% overlap. Color images available online at www.liebertpub.com/zeb

observed between 25 and 1200 Hz. Such irregular spectral shape contrasted with the ''smoother'' curve shape and more gradual energy decline found in natural habitats. Other studies have also reported higher sound energy <1000 Hz in artificial HS.[14,19,46] Such low-frequency noise is usually generated by water flows, ground vibrations, tank wall vibrations, and electrical pumps, whereas higher spectral peaks might result not only from oscillating and collapsing air bubbles and aeration, but also from electrical motors and water pumps.[14]

According to Lawrence and Mason,[47] to minimize noise sources in a zebrafish HS, the rack should contain dampeners on stands that support pumps or other vibratory and noisy equipment. According to the authors, the water treatment system should be isolated from the rack in a separate enclosed room. Our results showed that the system HS3 with a separate water treatment unit is significantly less noisy compared with the stand-alone systems (HS1 and HS2), although the noise levels were still well above the natural habitats with considerably more energy within the best hearing range of zebrafish.

Natural versus artificial soundscapes: potential effects on zebrafish hearing?

Zebrafish is an ostariophysan species with relatively wide frequency range detection (100–8000 Hz) and best hearing sensitivity at 600–1000 Hz.[22,24] This species is known to inhabit diverse freshwater habitats, ranging from stagnant water ponds to main river courses.[29] In this study we confirmed the presence of zebrafish in habitats that were considerably different in noise levels and spectral composition.

To evaluate potential hearing adaptation of the species to the various soundscapes, we considered auditory sensitivity curves previously determined from wild-type zebrafish lines.[22,25,48] We are aware of potential differences in hearing sensitivity between zebrafish in the wild compared with lines maintained in captivity and between specimens reared in different facilities. Therefore, we considered four audiograms obtained in distinct laboratories to show potential variability within the same species and due to technical differences in AEP measurements. Variation in audiograms between laboratories may also result from distinct background noise conditions and masking effects during AEP recordings, hence these data should be considered cautiously. Comparing audiograms of wild-type zebrafish lines with the various habitat noise spectra showed that this species is well adapted to all freshwater environments with probably some auditory masking in the fast-flowing river (SH). The noise spectral levels in SH were right below the species auditory thresholds between 800 and 2000 Hz in two of the audiograms reported.[22,33] Previous investigation on ostariophysan species belonging to the same family (Cyprinidae) have shown that habitat noise spectral levels that were just beneath the auditory thresholds within the most sensitive frequencies induced masking effects of about 9 dB (common carp *Cyprinus carpio*[7]) and 15 dB (topmouth minnow *Pseudorasbora parva*[49]).

Interestingly, the best hearing range of zebrafish (600–1000 Hz) matched a frequency interval where ambient noise spectra varied the most, but also a quieter window in the noisiest habitats located at 100–2000 Hz. Altogether, this suggests that, similar to other ostariophysan species,[7] zebrafish hearing sensitivity is well adapted to detect sounds in diverse freshwater habitats with different ecological characteristics (hydrology and substrate composition) and acoustic properties since the auditory thresholds are considerably above noise spectral levels or coincide with lower energy noise windows. However, the present study did not analyze the noise spectral levels during the rainy season, which might be considerably higher causing additional auditory masking effects. Future research should consider year-round changes of zebrafish natural soundscapes, as well as more details on habitat noise variability and relationship with hydrodynamic factors.

Furthermore, our results showed significant differences in noise levels and spectral composition between the soundscapes of natural habitats and zebrafish HS. Natural habitats were more variable in SPL and richer in abiotic and biotic noise sources compared with HS, which revealed a constant noise mostly generated by the pump and filtering equipment. Comparing noise spectral levels revealed differences up to 60 dB, especially below 1000 Hz. The artificial HS revealed spectral noise energy up to 22 dB above the species' best auditory thresholds, which most likely induces significant masking effects and maybe even hearing loss. Gutscher *et al.*[19] showed that 119 dB noise from external filters in aquaria induced auditory threshold shifts of up to 15–19 dB in *C. auratus* (noise level was about 8 dB above baseline audiogram). Wysocki and Ladich[32] reported that 130 dB of white noise evoked auditory thresholds shifts of up to 44 dB within the best hearing range of goldfish (*C. auratus*) (noise was up to 30 dB above baseline thresholds). Moreover, elevated noise levels may also induce hearing loss.[11,50] For example, Amoser and Ladich[50] exposed two otophysine species (goldfish *C. auratus*; and the catfish *Pimelodus pictus*) to 158 dB re 1 Pa white noise and identified significant hearing sensitivity loss within the species' best hearing range after 12–24 h of exposure (up to 26 dB in *C. auratus* and 32 dB in *P. pictus*). However, in this study, the noise level was higher compared with the SPL registered in the zebrafish HS and the species studied presented lower auditory sensitivities compared with zebrafish. Nevertheless, the effects of chronic exposure (since early ontogeny and across multigenerations) to noise levels found in typical artificial housing conditions remains to be investigated in otophysine species such as the zebrafish.

This study establishes an important ground for future research on the role of environmental noise shaping zebrafish hearing abilities in the wild, and highlights the importance of controlling noise conditions in fish HS. Elevated noise levels in zebrafish housing facilities may impact development of auditory organs and subsequently may affect studies on inner ear structure and function. Future work should investigate auditory masking effects of noise generated in zebrafish HS, as well as, potential hearing loss and physiological stress.

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