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Freshwater ecoacoustics: Listening to the ecological status of multi-stressed lowland waters

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ABSTRACT

A major challenge in water quality assessment is to identify suitable indicators to monitor and assess the effects of anthropogenic stressors on the ecological status of freshwater ecosystems. Passive acoustic monitoring is a novel approach that could potentially be used to detect invertebrate species and ecological processes such as dissolved oxygen dynamics in freshwater environments. The aim of the present study was to evaluate to what extent sounds can be used for water quality assessment. We performed a field study to relate acoustic indices to the intensity of several stressors, the invertebrate community composition and the dissolved oxygen dynamics in 20 temperate lowland streams and drainage ditches impacted to a varying degree by agricultural activities and discharges from waste water treatment plants. Our results showed that the recorded acoustic patterns were primarily associated with the fluctuation in dissolved oxygen saturation, while specific frequency bands could be related to the sound-producing invertebrate community. We observed that acoustic indices do not allow to detect the adverse effects of anthropogenic stressors on the invertebrate community composition, presumably due to the prevalence of Heteroptera which are relatively insensitive to stressors, but make a lot of sounds. A strong relation between acoustic indices and oxygen fluctuation indicate that passive acoustic monitoring may be used to estimate metabolism in water bodies. We suggest that the next step in freshwater ecoacoustics will be to precisely characterise each source of sound emitted during the processes of primary production, respiration and re-aeration, in order to distinguish these parameters. This may overcome some of the challenges encountered in the estimation of metabolism from diel dissolved oxygen curves.

1. Introduction

Freshwater ecosystems are commonly impacted by various anthropogenic activities, including agricultural activities and municipal wastewater treatment plant (WWTP) discharges (Burdon et al., 2019; Ormerod et al., 2010). These activities can result in a combination of stressors, such as excess nutrients, increased water temperatures, pesticides, pharmaceuticals, personal care products and a suite of other contaminants, which can cause substantial changes in the community composition and ecological processes of the impacted water bodies (Allan, 2004; Karr, 1999; Palmer and Febria, 2012). A major challenge in protecting and restoring these ecosystems is to identify cost-effective indicators to monitor, assess and evaluate the effects of these stressors on the ecological status of freshwater ecosystems (Bonada et al., 2006; Friberg et al., 2011).

The majority of ecological indicators used in water quality assessments are based on point-in-time measurements of the community structure of various organism groups (Boulton, 1999; Resh, 2008). Communities, especially of macroinvertebrates, are relatively diverse and can therefore be used to assess a variety of anthropogenic stressors (e.g. Clapcott et al., 2012). These structural-based indicators, however, do not capture the dynamic properties of freshwater ecosystems (Palmer and Febria, 2012). It has been argued that repeated measurements of ecological processes could be helpful to capture system dynamics employing functional-based indicators (Bunn, 1995). To this purpose, diel change in dissolved oxygen (DO) concentrations have

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been measured with loggers in the open channel to calculate ecosystem metabolism, where DO concentrations are associated with photosynthesis during the day and respiration at night (Odum, 1956; Young et al., 2008). Yet, these structural and functional based indicators require different sampling techniques. Thus the development of a sampling method that can monitor and assess structural and functional indicators with a single technique could improve the efficiency of water quality assessments.

A potential approach to combine structural and functional water quality assessment is to use passive acoustic monitoring, a method proposed by ecoacoustics that samples ambient sounds to tackle ecological questions (Farina and Gage, 2017; Sueur and Farina, 2015). This non-invasive and continuous monitoring method is emerging to address ecological questions in many ecosystems (Desjonquères et al., 2020b; Gibb et al., 2019; Linke et al., 2018; Sugai et al., 2019a, b). Some terrestrial studies have already managed to link acoustic patterns to the degrees of human influence (Burivalova et al., 2018; Buxton et al., 2018a), however, only a limited number of studies have applied ecoacoustic monitoring in freshwater environments (but see Desjonquères et al., 2015; Karaconstantis et al., 2020; Linke et al., 2020).

Environmental sounds can emanate not only from sound producing species, whose presence and activity depends on local ecological conditions, but also from ecological processes and anthropogenic activities (Linke et al., 2018). In freshwater environments, there are four main taxa known for producing sounds: amphibians, crustaceans, fish and insects (Desjonquères et al., 2020a). In temperate zones, most of the sounds recorded in freshwater environments appear to be linked to insects belonging to the insect orders Heteroptera, Coleoptera, Trichoptera and Odonata, Heteroptera and more specifically the family Corixidae include the majority of soniferous species (Aiken, 1985; Desjonquères, 2016; Desjonquères et al., 2018). Sounds linked to ecological processes are likely due to a combination of primary production through plant photosynthesis as well as the decomposition of organic matter by microbial activity (Linke et al., 2018). Although all these species and processes can potentially be detected and monitored in freshwater environments, the knowledge on species- and process-specific sounds is still emerging, making it unclear to what extent sounds can be used as a monitoring tool in water quality assessment strategies.

One of the main challenges of acoustic monitoring is to extract ecologically meaningful attributes of sounds and to link these sounds to ecosystem variables, such as community composition and ecological processes (Linke et al., 2018; Sueur and Farina, 2015). To address this challenge, acoustic indices, which are the equivalent of ecological diversity indices based on sound rather than on the number of species sampled, have been developed (Sueur et al., 2014). Such acoustic indices compute specific features of the sound spectrum (frequency representation) or waveform (temporal representation), which are thought to represent meaningful information about the ecosystem (Gage et al., 2017).

The aim of the present study was, therefore, to evaluate the potential of passive acoustic monitoring in freshwater environments by relating acoustic patterns to ecosystem structure and function in water bodies along a gradient of anthropogenic stress. We hypothesized that different acoustic indices relate to different structural and functional aspects of freshwater ecosystems. To test this hypothesis, we conducted a field study in 20 temperate lowland streams and drainage ditches throughout the Netherlands. First, we tested to what extent the invertebrate community reflected the intensity of the measured stressors including dissolved oxygen dynamics to confirm their use in water quality assessment. Then, we assessed if the sound-producing invertebrate community also reflected the same relationship to these stressors and dissolved oxygen dynamics. Finally, to evaluate the potential of passive acoustic monitoring in freshwater ecosystems, we correlated the acoustic indices to the measured stressors, the invertebrate community composition, the sound-producing invertebrate community composition and the dissolved oxygen dynamics.

2. Material and methods

2.1. Study outline

The 20 study sites were surrounded by varying degrees of agricultural land use in the riparian zone and WWTP effluent (Supplementary material 1). The sites had a comparable width (mean ± sd = 4.5 ± 1.8 m), depth (0.8 ± 0.2 m) and flow velocity (5.3 ± 6.8 cm/s). At each site, we measured nutrient concentrations, water temperature and proxies for pesticides, pharmaceuticals and personal care products to estimate the intensity of the stress originating from the agricultural activities and the WWTP discharges. As structural and functional indicators, we determined invertebrate community composition and dissolved oxygen dynamics, respectively. At all study sites, we recorded the underwater sounds from which we calculated acoustic indices. The study was conducted between August 20th and October 23th 2018. We chose this period, as the dissolved oxygen dynamics are most distinct during this period (Van der Lee et al., 2018) and it is likely to be within the reproductive period of several aquatic insect species (Jansson, 1974). All of this is expected to result in the most distinct sound patterns between sites. Stressors, dissolved oxygen dynamics and acoustics data was collected during the first six weeks of the sampling period, while invertebrate samples were collected during the last two weeks of the sampling period to avoid disturbing the acoustic sampling.

2.2. Stressors

Nutrient concentrations were measured by collecting a weekly surface water grab sample at each site for six weeks. The samples were filtered over a 1.2 µm filter and analysed for total dissolved nitrogen (TDN) and orthophosphate (PO_4-P) on a continuous flow analyser (SAN ++ system, Skalar Analytical B.V., Breda, The Netherlands). The mean nutrient concentrations over the six weeks were calculated for further analysis.

Water temperature (°C) was measured every ten minutes for six weeks with HOBO® Temperature/Light loggers UA-002-64 (Onset Computer Corporation, Bourne, MA, USA). At each study site, the loggers were placed in the mid-channel, 15 cm under the water surface. The mean water temperature over the six weeks was calculated for further analysis.

Proxies for pesticides, pharmaceuticals and personal care products were derived from bioassays subjected to passive sampler extracts. To this end, polar organic chemical integrative samplers (POCIS) containing 200 mg of Oasis hydrophilic-lipophilic balance sorbent were used (Waters, MA, USA; Alvarez et al., 2004). At each site, four POCIS were deployed for six weeks in the middle of the water column to absorb polar compounds from the surface water. After field exposure, the POCIS extracts were prepared and pooled for further analysis (details in Supplementary material 2). POCIS extracts were subjected to three in vitro chemical activated luciferase gene expression (CALUX®) bioassays at the BioDetection Systems laboratories (Amsterdam, the Netherlands), including the Estrogen receptor (ERα), the androgen receptor antagonism (anti-AR) and the progesterone receptor antagonism (anti-PR) CALUX assays. The activity of the extracts were expressed as bio-analytical equivalents of the corresponding reference compounds and divided by the effect-based trigger (EBT) value of each assay to obtain a measure of the ecotoxicological risk caused by the bioactive compounds present at each site (Brion et al., 2019; Escher et al., 2018). We considered ERα risk as a proxy for the presence of pharmaceuticals and personal care products and the mean of anti-AR and the anti-PR risks as a proxy for the presence of pesticides in the surface waters (Pieterse et al., 2015; Välitalo et al., 2016). Each stressor variable was scaled to a standard deviation of one and centred at its mean for further analysis.

Table 1
Invertebrate genera collected in this study that produce sound. Sound production in freshwater invertebrates was reviewed by Desjonquères (2016).

Order	Family	Genus
Trichoptera	Hydropsychidae	*Hydropsyche*
Coleoptera	Dytiscidae	*Cybister, Dytiscus*
	Haliplidae	*Haliplus*
	Hydrophilidae	*Anacaena, Enochrus, Helophorus*
Heteroptera	Corixidae	*Callicorixa, Corixa, Cymatia, Hesperocorixa, Micronecta, Paracorixa, Sigara*
	Nepidae	*Nepa, Ranatra*
	Pleidae	*Plea*
	Naucoridae	*Ilyocoris*
Decapoda	–	–

2.3. Invertebrate community composition

Six invertebrate samples were collected at each site on a single occasion. Three subsamples were taken with a pond net (1 mm mesh size, 25 cm width) that was swept over a length of 0.5 m of submerged vegetation, while the other three subsamples were taken with the same net swept over the top layer of the sediment. The samples were stored overnight at 4 °C with oxygen supply, washed over 1 mm and 250 µm sieves, sorted alive and preserved in 70% ethanol until identification. Overall, a total of 33,298 individuals belonging to 106 invertebrate taxa were collected. Invertebrates were identified to the genus level with a few exceptions, specifically Oligochaeta (order), Hydracarina (order) and Diptera (family). Corixidae were, if possible, further identified to the species level, as they are the family with the highest number of sound-producing species (Aiken, 1985). In total, 26 of the identified taxa are known for producing sounds (Table 1). The sum of taxon abundance for the six replicate invertebrate samples per site was log10(x + 1) transformed before further analysis to minimize the effect of high density taxa.

2.4. Dissolved oxygen dynamics

Dissolved oxygen (DO) concentrations (mg/L) were measured with optical HOBO® Dissolved Oxygen loggers U26-001, protected by the antifouling protective guard U26-GUARD-2 (Onset Computer Corporation, Bourne, MA, USA). Following Van der Lee et al. (2018) measurements were taken every ten minutes for six consecutive days in the mid-channel, 15 cm under the water surface. This was repeated three times at each site during the six week period, rotating weekly between the sites. Percent DO saturation was calculated from the DO concentrations and temperature, assuming 0‰ salinity and 1 atm barometric pressure, using DOTABLES developed by the U.S. Geological Survey (2011) for further analysis (Van der Lee et al., 2018). The mean DO saturation was calculated per 10 min time step over the 18 measurement days. The DO dynamics were represented by the mean DO saturation, while the fluctuation was calculated as the maximum values minus the minimum values.

2.5. Acoustic sampling

The underwater sounds were monitored with nine autonomous recording platforms consisting of a HTI-96 hydrophone (flat frequency response between 20 Hz and 40 kHz, High Tech Inc., Long Beach, MS, USA) connected with a 20 m cable to one channel of an autonomous SM2 audio recorder (Wildlife Acoustics, Maynard, MA, USA). A single SM2 recorder connected to a hydrophone was set up at each site to record uncompressed audio files (in the wav format) at a 44.1 kHz sampling frequency and a 16 bit digitization depth. The hydrophones were placed next to the dissolved oxygen sensors 15 cm below the water surface, with their piezoelectric element directed downward toward the sediment. The recording schedule was set to one minute every ten minutes, 24 h a day for six consecutive days. This was repeated two times at each site during the six week period, rotating between the sites. There was a two to three week interval between the first and second recording week.

To ensure the quality of the recordings used for the analysis, two randomly selected recordings per day were systematically examined by listening to the recording and inspecting the spectrogram. Due to one malfunctioning hydrophone, the data obtained with this hydrophone (5652 recordings) was excluded from our analyses. Recordings with high levels of anthropogenic noise were identified in one site and this data was also excluded (1792 recordings). We were not able to identify and remove occasional anthropogenic noise, however we expect it to have a negligible effect on our results. As rain can introduce unwanted noise and bias acoustic analyses, we also removed recordings collected when it was raining. Rain periods were assessed using the closest Royal Netherlands Meteorological Institute (KNMI) meteorological station (Supplementary material 1), resulting in the removal of 2879 recordings. This way, we obtained a final dataset of 26,989 recordings with an average of 1350 ± 575 (mean ± SD) one-minute recordings for each site (min: 209; max: 2095).

2.6. Acoustic analyses

The systematic examination of the sound recordings also allowed us to identify the specific frequency bands in which the main acoustic patterns occurred. This way, we identified three frequency bands which appeared to delimit most types of sounds: 0–2 kHz, 2–7 kHz and 7–22.05 kHz (Fig. 1). For each of these three bands, we computed three acoustic indices: the Acoustic Complexity Index (*ACI),* the Spectral Entropy (H_f), and the amplitude measured as the sum of raw amplitude from the spectrogram (*Amp)*. These three indices were chosen because they are indicative of different aspects of the soundscape (Buxton et al., 2018b; Towsey et al., 2018) and they have been used previously in some of the first ecoacoustic studies in freshwater environments (Desjonquères et al., 2015; Karaconstantis et al., 2020; Linke and Deretic, 2020). *ACI* is a measure that calculates the average difference of spectral amplitude between time windows (Pieretti et al., 2011). *Hf* is analogous to the Shannon entropy index from community ecology. Instead of species probability of presence, *Hf* uses the amplitude of each frequency bin in the mean spectrum (Sueur et al., 2008). This index thus yields a measure of the evenness of the probability mass function.

As sound is variable and dynamic over time it was recorded over multiple full days, which raises the issue of temporal autocorrelation and pseudo-replication (Desjonquères et al., 2020a). So far most research in ecoacoustics did not take into account such considerations, even though it may impact on the statistics and subsequent interpretation of the results. In this study, we propose a solution to this problem by employing Functional Data Analysis (FDA), which allows to represent a set of temporal data points as a single continuous mathematical function (Ramsay et al., 2009). Here, we used this method to describe and model the daily variations in each acoustic index at each site. To represent the smooth variation of the acoustic indices over a day, we calculated the mean values per ten minute time step resulting in 144 mean values per index per site. To obtain a representation of the daily variation for each site, we used 140 spline bases of order four and a smoothing parameter of 10^4. This specific smoothing parameter was chosen to optimise the trade-off between degrees of freedom and generalised cross validation (Ramsay et al., 2009). The modes of variation between the sites were then displayed in a Functional Principal Component Analysis (FPCA) for each acoustic index (Ramsay et al., 2009). This way, we obtained the coordinates of each site in a functional FPCA space. In such a FPCA, each axis represents the maximum variance between the sites in the shape of the splines. The scores for the first FPCA axis (FPC1) explained more than 67% of all acoustic indices and was used in subsequence analyses.

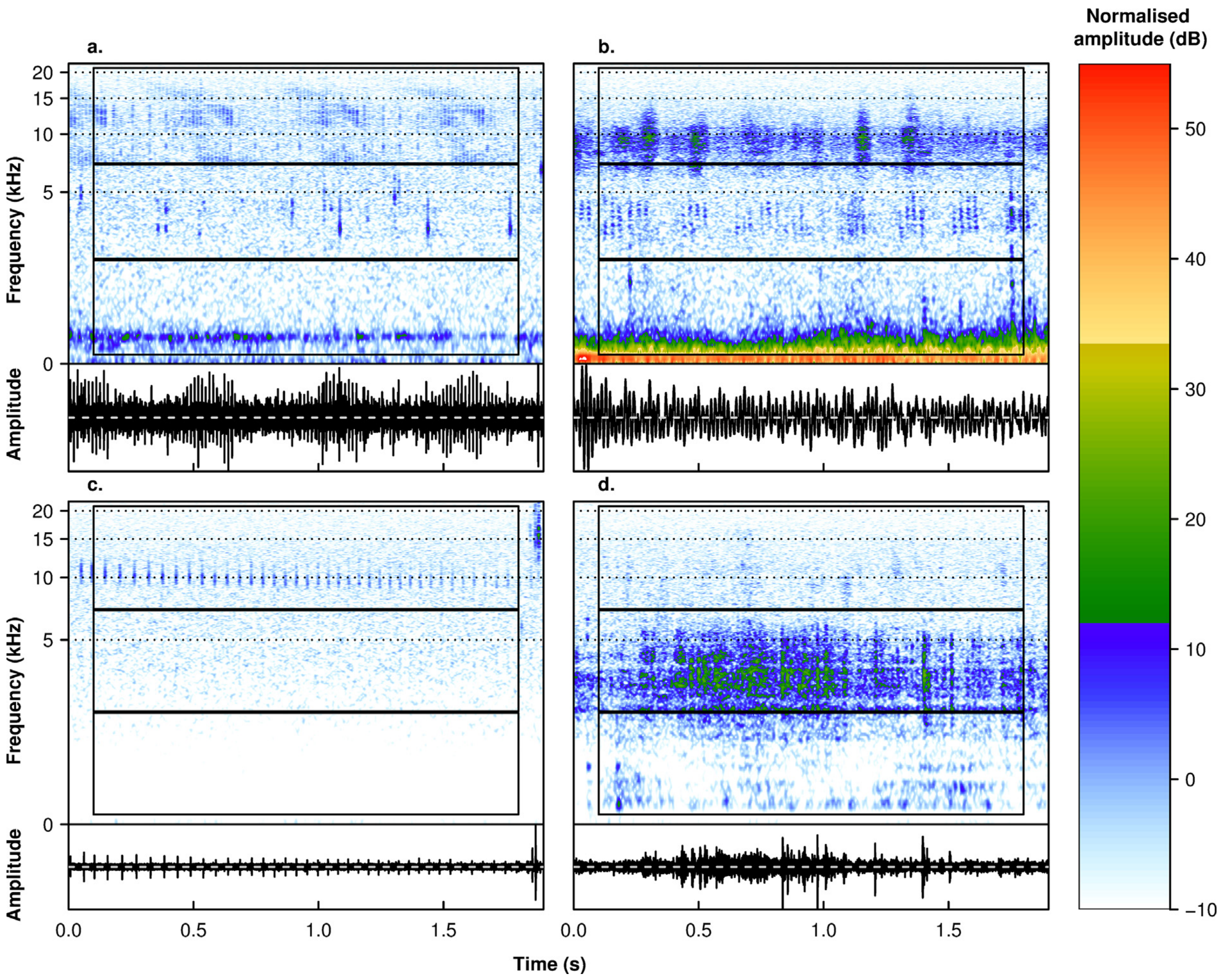

Fig. 1. Example of the main acoustic patterns in the recordings with the three frequency bands indicated that delimit most types of sounds: 0–2 kHz, 2–7 kHz and 7–22.05 kHz. (a) Recording from site 2 at 1:50 pm on August 25th showing the sounds of gusts of wind and continuous low frequency sounds potentially corresponding to photosynthesis (0–2 kHz), ticking and bubble sounds (2–7 kHz and 7–22.05 kHz); (b) Recording from site 8 at 6:00 am on August 24th showing the sounds of gusts of wind and continuous low frequency sounds potentially corresponding to photosynthesis (0–2 kHz), Corixidae sounds (2–7 kHz) and *Micronecta* sounds (7–22.05 kHz); (c) Recording from site 12 at 5:40 pm on August 28th showing ticking sounds (7–22.05 kHz); (d) Recording from site 8 at 14:40 pm on September 16th showing anthropogenic noise (2–7 kHz). Information on the sites in Supplementary material 1. The selected sound recordings can be found in Supplementary material 3–6.

2.7. Statistical analysis

To assess whether the sound-producing invertebrate community represents the same indication of the water quality as the entire community, we tested to what extent the separate unconstrained ordination (PCA) of the invertebrate taxa, both of the entire community and of only the sound-producing community, related to the stressors and dissolved oxygen dynamics. Then, to evaluate the potential of passive acoustic monitoring, we correlated the FPC1 of each acoustic index to a separate unconstrained ordination (PCA) of the measured stressors, the entire invertebrate community composition, the sound-producing invertebrate community composition and the dissolved oxygen dynamics. Significant relations between each acoustic index and each PCA was tested using a 999 permutation process. Significant vectors ($p < 0.05$) for acoustic indices were plotted on the ordination to show the correlation with the ordination configuration.

All analyses were performed in R (R Core Team, 2015; v. 3.6.0) using the *seewave* package to compute the acoustic indices (Sueur et al., 2018; v. 2.1.3), the *fda* package to compute the FPCA analysis (Ramsay et al., 2014; v. 2.4.8) and the package *vegan* to compute the PCA and to fit the acoustics indices on the ordinations (Oksanen et al., 2013; v. 2.5-6).

3. Results

The acoustic recordings contained various sound types associated with different processes including sound production by invertebrates, ticking and bubble sounds, wind and anthropogenic noise (Fig. 1, Supplementary material 3–6). These sound types were in most cases confined to specific frequency bands (0–2, 2–7 and 7–22.05 kHz). The daily variation of acoustic indices depended on the index, the site and the frequency band considered (Fig. 2). Some of the indices, such as $Hf_{0–2}$ and $Amp_{0–2}$, had similar temporal patterns with a maximum or minimum in the afternoon (12:00–18:00), but showed strong differences in the magnitude of the daily peaks. While others, like $Amp_{2–7}$, showed less differences in the magnitude of daily variation but higher differences in temporal patterns. Finally other indices, such as $Hf_{2–7}$, $Hf_{7–22}$ or $Amp_{2–7}$ differed both in magnitude of daily variations and in temporal patterns, with in some sites a secondary peak of activity at night.

In terms of the stressors, the entire invertebrate community showed significant correlations with orthophosphate concentrations, the proxies for pesticides and pharmaceuticals and the fluctuation in dissolved oxygen saturation (Fig. 3a, Table 2). The sound-producing invertebrate community only correlated significantly to the mean water temperature (Fig. 3b, Table 2).

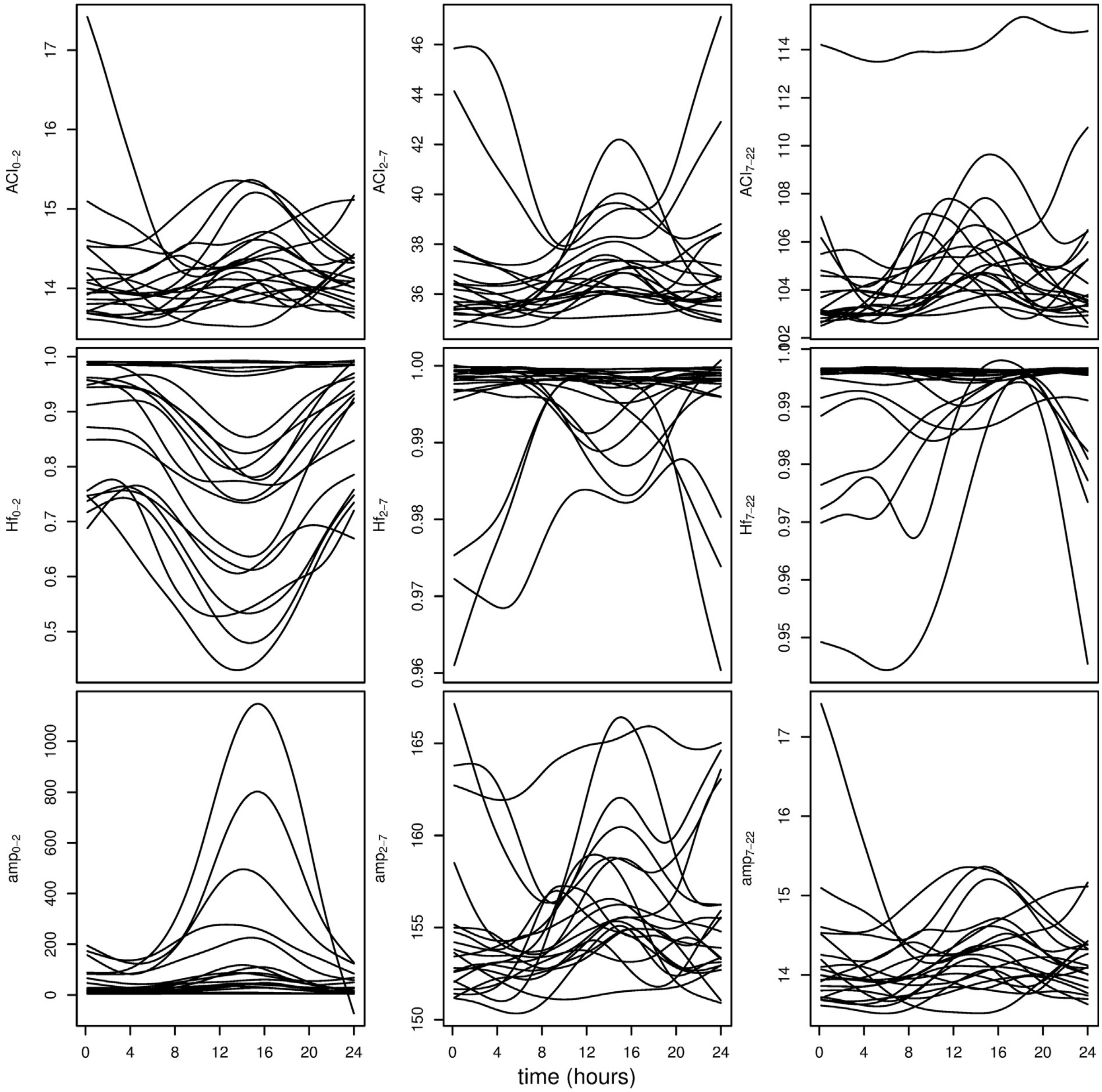

Fig. 2. FDA representation of the smooth variation of the acoustic indices over a single day. Each curve shows the FDA representation for a frequency band of an acoustic index in a site. The curves were obtained using a spline basis and a smoothing parameter. Notation: ACI_{0-2} corresponds to the *ACI* index over the 0–2 kHz frequency band.

In terms of the acoustic indices, the intensity of the stressors only correlated significantly to Hf_{0-2} (Fig. 4a, Table 3). Similarly to the stressors, the entire invertebrate community also correlated significantly with this acoustic index, as well as to Amp_{0-2} (Fig. 4b, Table 3). The sound-producing invertebrate community composition correlated significantly with *ACI* and *Hf* in the frequency band 2–7 kHz as well as $Amp_{7-22.05}$ and ACI_{0-2}. The loading scores for $Amp_{7-22.05}$ and ACI_{0-2} were in the same direction as the loading score of *Micronecta* on PC2 (Fig. 4c, Table 3). The dissolved oxygen saturation correlated significantly with all acoustic indices, except for ACI_{0-2} and Amp_{2-7} (Fig. 4d, Table 3). All arrows were mainly associated with the y-axis, representing fluctuations in dissolved oxygen. The strongest correlation was found with Amp_{0-2} ($R^2 = 0.52$, $p = 0.001$).

4. Discussion

The present study tested the relationship between acoustic patterns and the intensity of various anthropogenic stressors, invertebrate community composition and dissolved oxygen dynamics in temperate lowland streams and drainage ditches under a gradient of anthropogenic stress from agricultural activities and WWTP discharges. Our results showed that the acoustic patterns were primarily associated with the composition of the sound-producing invertebrate community and the fluctuation in dissolved oxygen saturation. Below we discuss to what extent these results corroborate the utility of passive acoustic monitoring in water quality assessment.

The sound-producing invertebrate community composition correlated to two acoustic indices in the frequency band 2–7 kHz (*ACI* and *Hf*), while the presence of *Micronecta* appeared to be associated with the high frequency band ($Amp_{7-22.05}$). This is coherent with the findings from previous studies showing that sounds produced by soniferous aquatic insects are concentrated within the 5–6.5 kHz range (Aiken, 1982), while *Micronecta* is known to generate a high-pitch sound with a dominant frequency in the 7–12 kHz band (Desjonquères et al., 2020b; Sueur et al., 2011). Indeed a wide diversity of invertebrates produce underwater sounds (Aiken, 1985; Desjonquères, 2016; Desjonquères

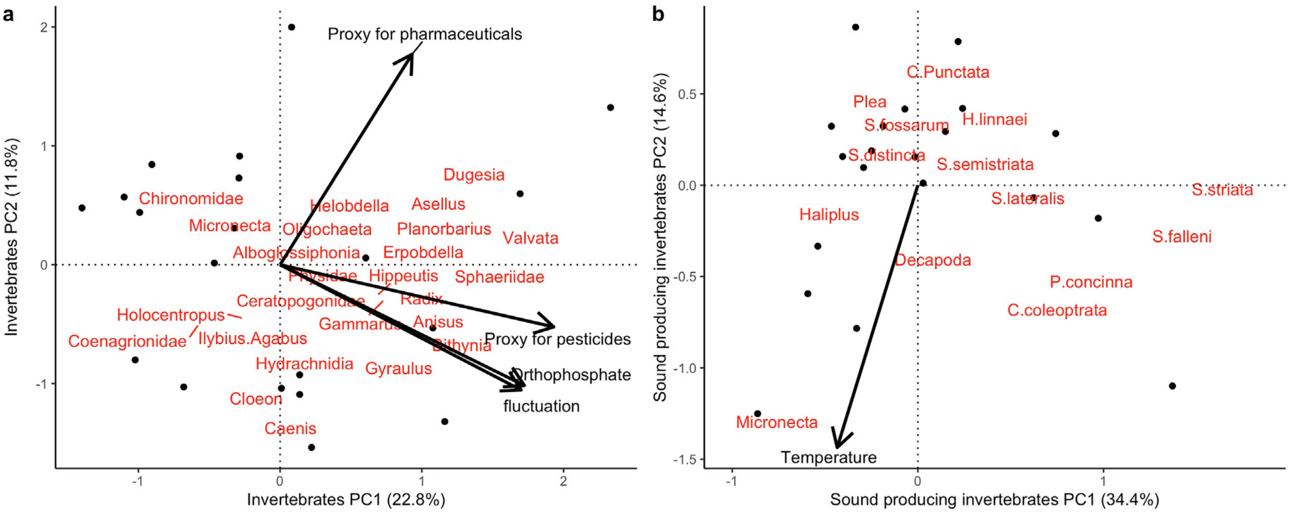

Fig. 3. Relations between the stressors, dissolved oxygen dynamics and the ordination of a) entire invertebrate community composition and b) sound-producing invertebrate community composition. Only significant vectors ($p < 0.05$) are plotted (details in Table 2). Taxa shown are prioritized on abundance.

Table 2
Relations between the stressors and dissolved oxygen dynamics and the ordination of the invertebrate communities (all taxa and only sound-producing taxa), as squared correlation coefficient (R^2) and significance (*p*) permutation-tested using 999 randomizations (N = 20).

	All invertebrate taxa		Sound-producing invertebrate taxa	
	R^2	p	R^2	p
Total dissolved nitrogen	0.08	0.541	0.204	0.148
Orthophosphate	0.31	**0.038**	0.003	0.982
Temperature	0.11	0.403	0.431	**0.006**
Proxy for pesticides	0.29	**0.049**	0.025	0.782
Proxy for pharmaceuticals	0.46	**0.010**	0.132	0.260
Mean dissolved oxygen	0.07	0.551	0.010	0.938
Fluctuation dissolved oxygen	0.40	**0.011**	0.087	0.482

et al., 2020a) and the family Corixidae is considered to be one of the main emitters of sounds in (shallow) freshwater environments (Desjonquères et al., 2018). Moreover, the daily pattern of these indices showed a distinct second peak at night, corroborating previous findings that Heteroptera chorus peak at 2 a.m. (Linke et al., 2020).

The acoustic indices were, however, not related to the intensity of the measured stressors. While the entire invertebrate community composition indicated several of the anthropogenic stressors, including nutrients, pollutants and dissolved oxygen fluctuation, the sound-producing taxa only related significantly to temperature. This corroborates the common use of invertebrate community composition in water quality assessment, as they are a diverse group reflecting a wide range of sensitivity to anthropogenic stressors (Resh and Rosenberg, 1993). The lack of a relationship between the measured stressors and the acoustic indices may be due to the fact that the majority of sound-producing invertebrate taxa occurring in these lowland waters belong to the order of Heteroptera, which are generally considered moderately tolerant to chemical stressors, such as nutrient loading and organic toxicants (Lock et al., 2013; Von der Ohe and Liess, 2004). Previous studies rather related their presence to the habitat structure of water bodies, such as vegetation coverage (Dias-Silva et al., 2010; Olosutean and Ilie, 2013), which was not included in our study. This was confirmed by Desjonquères et al. (2018) who showed that when vegetation density was included as part of the studied gradient, the acoustic community could indeed be related to the entire invertebrate community. So, even though the acoustic indices could represent the sound-producing invertebrate community, they may have limited value as indicators in water quality assessment, as they did not represent the impact of different anthropogenic stressors.

Almost all acoustic indices (7 out of 9) were associated with the daily fluctuation in dissolved oxygen saturation and many of them showed a peak in the afternoon, which is a pattern typically observed for dissolved oxygen saturation in these water bodies. Similarly, Felisberto et al. (2015) observed that acoustic patterns in low (0.4–0.8 kHz) and medium (1.5–3.5 kHz) frequency bands followed the same diel cycle as measured by dissolved oxygen loggers in a marine environment. Interestingly, these frequency bands are similar to the ones for which high correlations values were observed in this study. Dissolved oxygen saturation in the water column is affected by 1) the release of oxygen by photosynthetic primary producers during the day, 2) the uptake of oxygen through respiration by all organisms and 3) the exchange of oxygen with the air (i.e. re-aeration) (Odum, 1956). Previous studies have shown that each of these processes produces sounds. Specifically, Kratochvil and Pollirer (2017) reported that an aquatic plant, *Elodea canadensis*, emits short sound pulses with a wide frequency band as it produces and releases oxygen bubbles in the water. Freeman et al. (2018) observed similar results for marine macroalgae and argued that these sounds may thus be used as an indicator for photosynthetic activity. The respiration by microorganisms decomposing organic matter (both aerobically and anaerobically) is also suspected to produce ticking sounds, as gas bubbles are formed and expelled (Felisberto et al., 2015; Linke et al., 2018). Lastly, Morse et al. (2007) were able to relate sounds at the water-air interface to re-aeration rates.

Our findings, along with these studies, indicate that acoustic indices could be used to estimate metabolism in water bodies, which may subsequently be used in water quality assessment. Passive acoustic monitoring may even overcome certain challenges encountered in the estimation of metabolism from diel dissolved oxygen curves, such as the possibility to split up different components if different processes emit different acoustic patterns, the ability to estimate re-aeration rates and the inclusion of anaerobic respiration (Staehr et al., 2012). Future research should focus on the selection of suitable frequency bands or the detection of specific acoustic patterns that are not sensitive to confounding factors, affecting the acoustic patterns emitted by metabolism-related processes, such as the sounds of invertebrates, surface agitation due to wind and the influence of water movement on bubble formation and retention (Felisberto et al., 2015; Freeman et al., 2018). This may be achieved with a combination of laboratory and field studies identifying the acoustic patterns emitted by specific sources of underwater sounds.

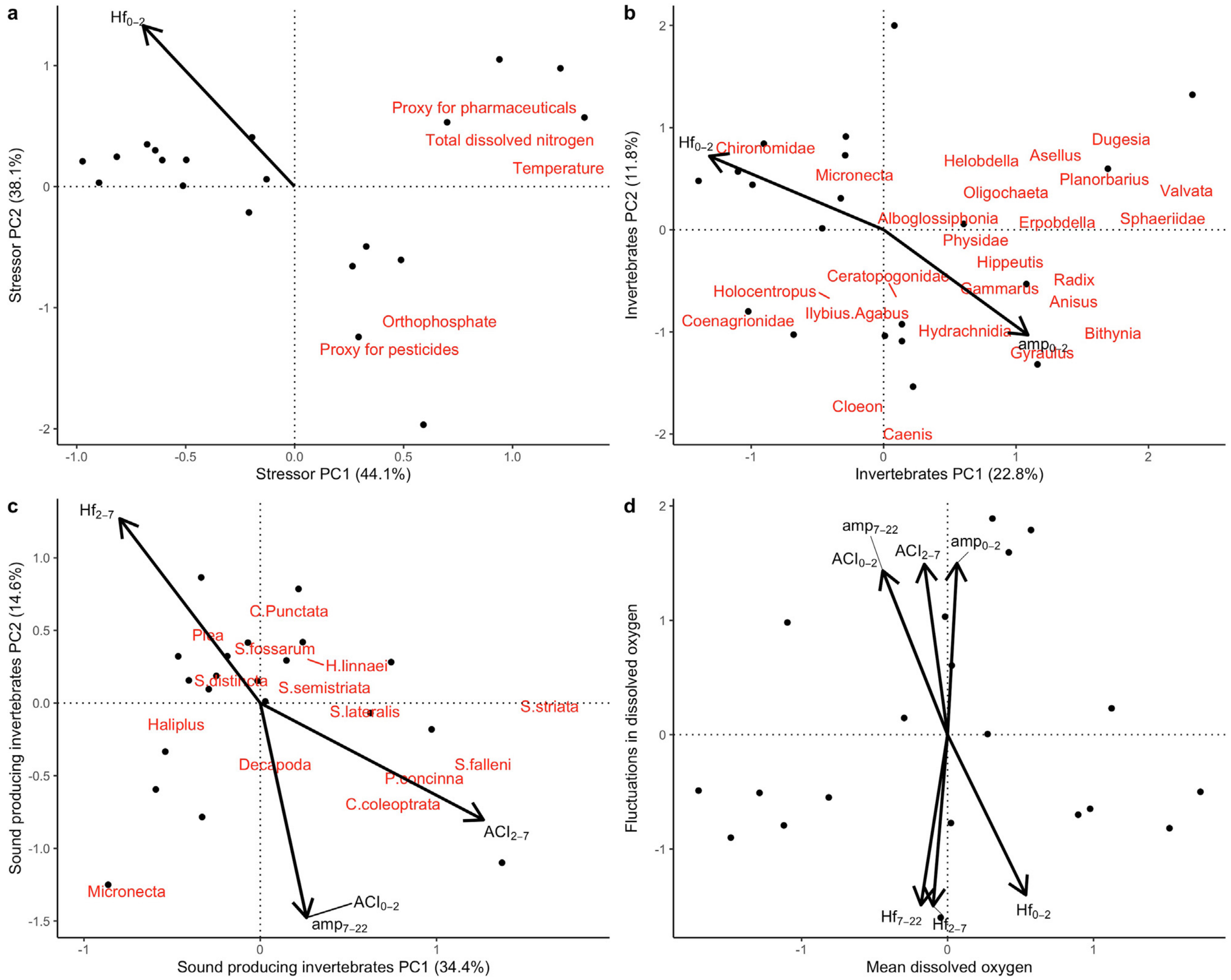

Fig. 4. Relations between the acoustic indices and the ordination of different environmental variables, including a) stressors, b) dissolved oxygen dynamics, c) entire invertebrate community composition and d) sound-producing invertebrate community composition. Only significant vectors ($p < 0.05$) are plotted (details in Table 3). Taxa shown are prioritized on abundance.

Table 3
Relations between the acoustic indices and the ordination of different environmental variables as squared correlation coefficient (R^2) and significance (p) permutation-tested using 999 randomizations (N = 20).

		Stressors		All invertebrate taxa		Sound-producing invertebrate taxa		Dissolved oxygen saturation	
		R^2	p	R^2	p	R^2	p	R^2	p
ACI	0–2	0.08	0.509	0.07	0.558	0.35	**0.025**	0.31	**0.044**
	2–7	0.11	0.346	0.20	0.143	0.41	**0.031**	0.37	**0.017**
	7–22.05	0.05	0.588	0.07	0.532	0.00	0.969	0.06	0.656
Hf	0–2	0.30	**0.048**	0.54	**0.004**	0.15	0.247	0.47	**0.009**
	2–7	0.11	0.326	0.16	0.236	0.38	**0.019**	0.44	**0.006**
	7–22.05	0.18	0.164	0.06	0.557	0.24	0.096	0.46	**0.004**
Amp	0–2	0.21	0.108	0.40	**0.015**	0.13	0.279	0.52	**0.001**
	2–7	0.02	0.815	0.21	0.127	0.09	0.425	0.20	0.149
	7–22.05	0.08	0.509	0.07	0.558	0.35	**0.025**	0.31	**0.044**

In conclusion, the presently employed acoustics indices allowed the detection of sound-producing invertebrate taxa as well as the fluctuation in dissolved oxygen saturation. In terms of water quality assessment, the acoustic indices poorly indicated the intensity of the anthropogenic stressors compared to the traditional method which samples invertebrate communities with a net, presumably due to the dominance of the relatively insensitive Heteroptera in the sound-producing community. In contrast, the strong relation between acoustic indices and oxygen fluctuation indicated that passive acoustic monitoring may be used to estimate metabolism in these water bodies. The knowledge of these sounds is still emerging, we therefore suggest that the next step in freshwater ecoacoustics is to precisely characterise the sounds individually emitted by photosynthesis, respiration and re-aeration, so these processes can be distinguished. This would greatly enhance the potential of ecoacoustics as a monitoring tool in freshwater environments.

Author contributions

GHvdL, CD and PFMV conceived the ideas and designed methodology; GHvdL conducted the field work, collecting the data and identifying invertebrate samples; CD and PFMV participated in the field work; CD and GHvdL analysed the data and led the redaction of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106252.

References

Aiken, R.B., 1985. Sound production by aquatic insects. Biol. Rev. 60, 163–211.

Aiken, R.B., 1982. Shallow-water propagation of frequencies in aquatic insect sounds. Can. J. Zool. 60, 3459–3461. https://doi.org/10.1139/z82-436.

Allan, J.D., 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annu. Rev. Ecol. Evol. Syst. 35, 257–284.

Alvarez, D.A., Petty, J.D., Huckins, J.N., Jones-Lepp, T.L., Getting, D.T., Goddard, J.P., Manahan, S.E., 2004. Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. Environ. Toxicol. Chem.: Int. J. 23, 1640–1648.

Bonada, N., Prat, N., Resh, V.H., Statzner, B., 2006. Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. Annu. Rev. Entomol. 51, 495–523.

Boulton, A.J., 1999. An overview of river health assessment: philosophies, practice, problems and prognosis. Freshw. Biol. 41, 469–479.

Brion, F., De Gussem, V., Buchinger, S., Hollert, H., Carere, M., Porcher, J.-M., Piccini, B., Féray, C., Dulio, V., Könemann, S., 2019. Monitoring estrogenic activities of waste and surface waters using a novel in vivo zebrafish embryonic (EASZY) assay: comparison with in vitro cell-based assays and determination of effect-based trigger values. Environ. Int. 130, 104896.

Bunn, S.E., 1995. Biological monitoring of water quality in Australia: workshop summary and future directions. Aust. J. Ecol. 20, 220–227.

Burdon, F.J., Munz, N.A., Reyes, M., Focks, A., Joss, A., Räsänen, K., Altermatt, F., Eggen, R.I.L., Stamm, C., 2019. Agriculture versus wastewater pollution as drivers of macroinvertebrate community structure in streams. Sci. Total Environ. 659, 1256–1265.

Burivalova, Z., Towsey, M., Boucher, T., Truskinger, A., Apelis, C., Roe, P., Game, E.T., 2018. Using soundscapes to detect variable degrees of human influence on tropical forests in Papua New Guinea: tropical-forest soundscapes. Conserv. Biol. 32, 205–215. https://doi.org/10.1111/cobi.12968.

Buxton, R.T., Lendrum, P.E., Crooks, K.R., Wittemyer, G., 2018a. Pairing camera traps and acoustic recorders to monitor the ecological impact of human disturbance. Global Ecol. Conserv. 16, e00493. https://doi.org/10.1016/j.gecco.2018.e00493.

Buxton, R.T., McKenna, M.F., Clapp, M., Meyer, E., Stabenau, E., Angeloni, L.M., Crooks, K., Wittemyer, G., 2018b. Efficacy of extracting indices from large-scale acoustic recordings to monitor biodiversity: acoustical monitoring. Conserv. Biol. 32, 1174–1184. https://doi.org/10.1111/cobi.13119.

Clapcott, J.E., Collier, K.J., Death, R.G., Goodwin, E.O., Harding, J.S., Kelly, D., Leathwick, J.R., Young, R.G., 2012. Quantifying relationships between land-use gradients and structural and functional indicators of stream ecological integrity. Freshw. Biol. 57, 74–90.

Desjonquères, C., 2016. Acoustic diversity and ecology of freshwater environments: exploration in temperate environments (PhD Thesis). Museum national d'histoire naturelle-MNHN, Paris.

Desjonquères, C., Gifford, T., Linke, S., 2020a. Passive acoustic monitoring as a potential tool to survey animal and ecosystem processes in freshwater environments. Freshw Biol 65, 7–19. https://doi.org/10.1111/fwb.13356.

Desjonquères, C., Rybak, F., Castella, E., Llusia, D., Sueur, J., 2018. Acoustic communities reflects lateral hydrological connectivity in riverine floodplain similarly to macroinvertebrate communities. Sci. Rep. 8. https://doi.org/10.1038/s41598-018-31798-4.

Desjonquères, C., Rybak, F., Depraetere, M., Gasc, A., Le Viol, I., Pavoine, S., Sueur, J., 2015. First description of underwater acoustic diversity in three temperate ponds. PeerJ 3, e1393. https://doi.org/10.7717/peerj.1393.

Desjonquères, C., Rybak, F., Ulloa, J.S., Kempf, A., Bar Hen, A., Sueur, J., 2020b. Monitoring the acoustic activity of an aquatic insect population in relation to temperature, vegetation and noise. Freshw. Biol. 65, 107–116. https://doi.org/10.1111/fwb.13171.

Dias-Silva, K., Cabette, H.S., Juen, L., De Marco Jr., P., 2010. The influence of habitat integrity and physical-chemical water variables on the structure of aquatic and semiaquatic Heteroptera. Zoologia 27.

Escher, B.I., Aït-Aïssa, S., Behnisch, P.A., Brack, W., Brion, F., Brouwer, A., Buchinger, S., Crawford, S.E., Du Pasquier, D., Hamers, T., 2018. Effect-based trigger values for in vitro and in vivo bioassays performed on surface water extracts supporting the environmental quality standards (EQS) of the european water framework directive. Sci. Total Environ. 628, 748–765.

Farina, A., Gage, S.H., 2017. Ecoacoustics: The Ecological Role of Sounds. John Wiley & Sons.

Felisberto, P., Jesus, S.M., Zabel, F., Santos, R., Silva, J., Gobert, S., Beer, S., Björk, M., Mazzuca, S., Procaccini, G., Runcie, J.W., Champenois, W., Borges, A.V., 2015. Acoustic monitoring of O2 production of a seagrass meadow. J. Exp. Mar. Biol. Ecol. 464, 75–87. https://doi.org/10.1016/j.jembe.2014.12.013.

Freeman, S.E., Freeman, L.A., Giorli, G., Haas, A.F., 2018. Photosynthesis by marine algae produces sound, contributing to the daytime soundscape on coral reefs. PLoS ONE 13, e0201766.

Friberg, N., Bonada, N., Bradley, D.C., Dunbar, M.J., Edwards, F.K., Grey, J., Hayes, R.B., Hildrew, A.G., Lamouroux, N., Trimmer, M., 2011. Biomonitoring of human impacts in freshwater ecosystems: the good, the bad and the ugly. Adv. Ecol. Res. Elsevier 1–68.

Gage, S.H., Towsey, M., Kasten, E.P., 2017. Analytical methods in ecoacoustics. Wiley, Oxford, UK, pp. 273–296.

Gibb, R., Browning, E., Glover-Kapfer, P., Jones, K.E., 2019. Emerging opportunities and challenges for passive acoustics in ecological assessment and monitoring. Methods Ecol. Evol. 10, 169–185. https://doi.org/10.1111/2041-210X.13101.

Jansson, A., 1974. Annual periodicity of male stridulation in the genus *Cenocorixa* (Hemiptera, Corixidae). Freshw. Biol. 4, 93–98. https://doi.org/10.1111/j.1365-2427.1974.tb00941.x.

Karaconstantis, C., Desjonquères, C., Gifford, T., Linke, S., 2020. Micro- and macro-variation in a chain of waterholes. Freshw. Biol. 65, 96–106.

Karr, J.R., 1999. Defining and measuring river health. Freshw. Biol. 41, 221–234.

Kratochvil, H.G., Pollirer, M., 2017. Acoustic effects during photosynthesis of aquatic plants enable new research opportunities. Sci. Rep. 7, 44526. https://doi.org/10.1038/srep44526.

Linke, S., Decker, E., Gifford, T., Desjonquères, C., 2020. Diurnal variation in freshwater ecoacoustics: implications for site-level sampling design. Freshw. Biol. 65, 86–95. https://doi.org/10.1111/fwb.13227.

Linke, S., Deretic, J., 2020. Ecoacoustics can detect ecosystem responses to environmental water allocations. Freshw. Biol. 65, 133–141. https://doi.org/10.1111/fwb.13249.

Linke, S., Gifford, T., Desjonquères, C., Tonolla, D., Aubin, T., Barclay, L., Karaconstantis, C., Kennard, M.J., Rybak, F., Sueur, J., 2018. Freshwater ecoacoustics as a tool for continuous ecosystem monitoring. Front. Ecol. Environ. 16, 231–238. https://doi.org/10.1002/fee.1779.

Lock, K., Adriaens, T., Van De Meutter, F., Goethals, P., 2013. Effect of water quality on waterbugs (Hemiptera: Gerromorpha & Nepomorpha) in Flanders (Belgium): results from a large-scale field survey. Ann. Limnologie-Int. J. Limnol. EDP Sci. 121–128.

Morse, N., Bowden, W.B., Hackman, A., Pruden, C., Steiner, E., Berger, E., 2007. Using sound pressure to estimate reaeration in streams. J. North Am. Benthol. Soc. 26, 28–37.

Odum, H.T., 1956. Primary production in flowing waters 1. Limnol. Oceanogr. 1, 102–117.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Package 'vegan.' Community ecology package, version 2.

Olosutean, H., Ilie, D.M., 2013. Are semi-aquatic bugs (Heteroptera: Nepomorpha) indicators of hydrological stability of permanent ponds? Aquatic Insects 35, 105–118.

Ormerod, S.J., Dobson, M., Hildrew, A.G., Townsend, C., 2010. Multiple stressors in freshwater ecosystems. Freshw. Biol. 55, 1–4.

Palmer, M.A., Febria, C.M., 2012. The heartbeat of ecosystems. Science 336, 1393–1394.

Pieretti, N., Farina, A., Morri, D., 2011. A new methodology to infer the singing activity of an avian community: the acoustic complexity index (ACI). Ecol. Ind. 11, 868–873. https://doi.org/10.1016/j.ecolind.2010.11.005.

Pieterse, B., Rijk, I.J.C., Simon, E., van Vugt-Lussenburg, B.M.A., Fokke, B.F.H., Van der Wijk, M., Besselink, H., Weber, R., van der Burg, B., 2015. Effect-based assessment of persistent organic pollutant and pesticide dumpsite using mammalian CALUX reporter cell lines. Environ. Sci. Pollut. Res. 22, 14442–14454.

RCore Team, 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramsay, J., Hooker, G., Graves, S., 2009. Functional Data Analysis with R and MATLAB. Springer New York, New York, NY.

Ramsay, J.O., Wickham, H., Graves, S., Hooker, G., 2014. FDA: Functional Data Analysis.

Resh, V.H., 2008. Which group is best? Attributes of different biological assemblages used in freshwater biomonitoring programs. Environ. Monit. Assess. 138, 131–138.

Resh, V.H., Rosenberg, D.M., 1993. Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall New York, NY, USA.

Staehr, P.A., Testa, J.M., Kemp, W.M., Cole, J.J., Sand-Jensen, K., Smith, S.V., 2012. The metabolism of aquatic ecosystems: history, applications, and future challenges. Aquat. Sci. 74, 15–29.

Sueur, J., Aubin, T., Simonis, C., Lellouch, L., Brown, E.C., Depraetere, M., Desjonqueres, C., Fabianek, F., Gasc, A., Kasten, E., 2018. Package 'seewave.'.

Sueur, J., Farina, A., 2015. Ecoacoustics: the ecological investigation and interpretation of environmental sound. Biosemiotics 8, 493–502. https://doi.org/10.1007/s12304-015-9248-x.

Sueur, J., Farina, A., Gasc, A., Pieretti, N., Pavoine, S., 2014. Acoustic indices for biodiversity assessment and landscape investigation. Acta Acust. United Acust. 100, 772–781. https://doi.org/10.3813/AAA.918757.

Sueur, J., Mackie, D., Windmill, J.F.C., 2011. So small, so loud: extremely high sound pressure level from a pygmy aquatic insect (Corixidae, Micronectinae). PLoS ONE 6, e21089. https://doi.org/10.1371/journal.pone.0021089.

Sueur, J., Pavoine, S., Hamerlynck, O., Duvail, S., 2008. Rapid acoustic survey for biodiversity appraisal. PLoS ONE 3, e4065. https://doi.org/10.1371/journal.pone.0004065.

Sugai, L.S.M., Desjonquères, C., Silva, T.S.F., Llusia, D., 2019a. A roadmap for survey designs in terrestrial acoustic monitoring. Remote Sens. Ecol. Conserv. rse2.131. https://doi.org/10.1002/rse2.131.

Sugai, L.S.M., Silva, T.S.F., Ribeiro, J.W., Llusia, D., 2019b. Terrestrial passive acoustic monitoring: review and perspectives. Bioscience 69, 15–25. https://doi.org/10.1093/biosci/biy147.

Towsey, M., Znidersic, E., Broken-Brow, J., Indraswari, K., Watson, D.M., Phillips, Y., Truskinger, A., Roe, P., 2018. Long-duration, false-colour spectrograms for detecting species in large audio data-sets. J. Ecoacoust. 2, IUSWUI.

Välitalo, P., Perkola, N., Seiler, T.-B., Sillanpää, M., Kuckelkorn, J., Mikola, A., Hollert, H., Schultz, E., 2016. Estrogenic activity in Finnish municipal wastewater effluents. Water Res. 88, 740–749.

Van der Lee, G.H., Verdonschot, R.C., Kraak, M.H., Verdonschot, P.F., 2018. Dissolved oxygen dynamics in drainage ditches along a eutrophication gradient. Limnologica 72, 28–31.

Von der Ohe, P.C., Liess, M., 2004. Relative sensitivity distribution of aquatic invertebrates to organic and metal compounds. Environ. Toxicol. Chem.: Int. J. 23, 150–156.

Young, R.G., Matthaei, C.D., Townsend, C.R., 2008. Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. J. North Am. Benthol. Soc. 27, 605–625.