Spatio-temporal heterogeneity in river sounds -disentangling micro-and macro-variation in a chain of waterholes Spatio-temporal sound patterns in a chain of waterholes

Chris Karaconstantis, Camille Desjonquères, Toby Gifford, Simon Linke

Spatio-temporal heterogeneity in river sounds – disentangling micro- and macro-variation in a chain of waterholes

Spatio-temporal sound patterns in a chain of waterholes

Chris Karaconstantis[1,*], Camille Desjonquères[2,3,*,†], Toby Gifford[4], Simon Linke[1]

[1] Australian Rivers Institute, Griffith University, Nathan, QLD, 4111, Australia

[2] Muséum national d'Histoire naturelle, Institut Systématique, Evolution, Biodiversité ISYEB, UMR 7205 CNRS MNHN UPMC EPHE, 45 rue Buffon, 75005 Paris, France

[3] Université Paris-Sud, CNRS UMR 8195 CNPS, Bâtiments 446, Université Paris-Sud, 91405 Orsay cedex, France

[4] SensiLab, Monash University, Caulfield, VIC 3045, Australia

[*] These authors contributed equally to this work

[†] Corresponding author: cdesjonqu@gmail.com, Tel.: +1 (414) 229-4214, Muséum national d'Histoire naturelle, 45 rue Buffon, 75005 Paris, France

Abstract

1. Passive acoustic monitoring is gaining momentum as a viable alternative method to surveying freshwater ecosystems. As part of an emerging field, the spatio-temporal replication levels of these sampling methods need to be standardized. However, in shallow waters, acoustic spatio-temporal patchiness remains virtually unexplored.

2. In this paper, we specifically investigate the spatial heterogeneity in underwater sounds observed within and between waterholes of an ephemeral river at different times of the day and how it could affect sampling in passive acoustic monitoring.

3. We recorded in the Einasleigh River, Queensland in August 2016, using a linear transect of hydrophones mounted on frames. We recorded four times a day: at dawn, midday, dusk and midnight. To measure different temporal and spectral attributes of the recorded sound, we investigated the mean frequency spectrum and computed acoustic indices.

4. Both mean frequency spectrum and index analyses revealed that the site and diel activity patterns significantly influenced the sounds recorded, even for adjacent sites with similar characteristics along a single river. We found that most of the variation was due to temporal patterns, followed by between-site differences, while within-site differences had limited influence.

5. This study demonstrates high spatio-temporal acoustic variability in freshwater environments, linked to different species or species groups. Decisions about sampling design are vital to obtain adequate representation. This study thus emphasizes the need to tailor spatio-temporal settings of a sampling design to the aim of the study, the species and the habitat.

Keywords: ecoacoustics, aquatic environments, passive acoustics, sampling design, ecological monitoring

Introduction

Traditional monitoring of freshwater ecological communities has major limitations: animals are subject to injuries or mortality with methods such as netting, trapping and electrofishing (Pidgeon, 2003); often, spatial and temporal variation cannot be obtained without many devoted hours of study (Goodman *et al.*, 2015); and uncommon or rare species are hard to account for (Dufrêne & Legendre, 1997; Ovaskainen & Soininen, 2011). Additionally, in low visibility areas, such as turbid rivers, visual inspections are often impracticable. One alternative approach that mitigates these issues is to monitor the sounds in the environment (Linke *et al.*, 2018b).

Passive acoustic monitoring (PAM) offers many benefits: it is non-invasive, user friendly, does not induce flight response due to observer presence, can be used in low visibility environments, and enables long term monitoring to assess seasonal variation (Gannon, 2008; Anderson, Rountree & Juanes, 2008). With recent technological advances, the collection and analysis of audio recordings is becoming more accessible to researchers. Dedicated automated analysis methods, such as automated signal recognisers (Towsey, Parsons & Sueur, 2014a) allow to process large quantities of audio recordings quickly. Spectral and temporal features of audio recordings can also be summarised by acoustic indices, analogous to those used in ecology (Sueur *et al.*, 2014; Phillips, Towsey & Roe, 2018). Just as species richness, diversity and Shanon entropy are single numerical values thought to measure relevant attributes of an ecosystem; acoustic richness, diversity and entropy of a recording can also be calculated to measure relevant attributes of soundscapes and ecosystems (Villanueva-Rivera *et al.,* 2011; Depraetere *et al.*, 2012). Although these indices forego species identification and are designed to quantify specific attributes of the soundscape (Farina & Gage, 2017), they can describe species-specific patterns if a species dominates a soundscape or a frequency band (Towsey *et al.*, 2018; Linke *et al.*, 2018a; Indraswari *et al.*, 2018). These advances and other major advantages make PAM a viable option in freshwaters. Indeed, the use of PAM is gaining traction as an ecological tool in this realm (Linke *et al.*, 2018b).

Sound is far less attenuated in water than air. Thus some marine mammals can be recorded from several km away (Risch *et al.*, 2014). However marine mammals produce extremely high amplitude, and low frequency sounds in the open ocean . Sounds of freshwater organisms (such as insects or fish) have lower amplitudes. An important proportion of freshwater environments, such as small ponds and streams, are shallow. In such environments, sound propagation is complex due to the reflection of sound at the bottom and surface of the water (Farcas, Thompson & Merchant, 2016). Sound propagation in freshwater environments may be even more complex due to the presence of vegetation, and to the diversity of sediment nature (e.g. soft and organic, sandy or rocky). The few studies on sound propagation in freshwater environments have shown that sound attenuates over less than a meter (Aiken, 1982) and that shallow waters act as high-pass filters, with the cut-off frequency getting lower as the water column gets deeper, according to the theory of waveguides (Forrest, Miller & Zagar, 1993).

Similarly to the spatial heterogeneity of species in the landscape, soundscapes are extremely variable (Gasc *et al.*, 2013; Parks, Miksis-Olds & Denes, 2014). Sound production as an animal behaviour features temporal variations such as diel and seasonal periodicity (Bohnenstiehl, Lillis & Eggleston, 2016). This diversity of schedule and spatial heterogeneity suggests that recording at single locations and for short periods might be unrepresentative of the overall soundscape. On the other hand multiple recordings (or an adequate duration of recording) may reveal underlying temporal and spatial patterns and better capture overall levels of diversity.

Only a few studies address spatio-temporal variation in freshwaters and their consequences for PAM (Linke *et al.*, 2018b a; Gottesman *et al.*, 2018). Therefore, there is a need to investigate how to design appropriate sampling protocols to account for the various sources of heterogeneity. Here we investigate the extent of spatio-temporal variations in a freshwater environment. Using PAM in four separated waterholes of an ephemeral river, our specific aims were to: 1. determine the extent of variation of underwater sound between nearby waterholes of the same river; 2. determine the extent of spatial variation of underwater sound within river waterholes; 3. estimate diurnal variation in underwater sounds. This variation has already been estimated in other studies (Desjonquères *et al.*, 2015; Linke *et al.*, 2018a) but was not previously compared to spatial variation; 4. compare variation due to spatial and temporal factors observed in underwater sounds; 5. interpret how these variations may affect acoustic assessments conducted with different sampling regimes and methods of analyses. We conclude by suggesting best practices and future research necessary to standardise PAM in freshwater environments. Although to estimate the exact sampling effort required, we would need to measure species-specific detection probability, this objective is beyond the scope of our study. In this study, we undertake the first step to standardising protocols: test whether there is significant spatio-temporal sources of variation and compare the relative contribution of different sources of variability.

Material and methods

Overview

To determine spatial acoustic differences between- and within-sites, we recorded underwater at four sites along a river. Each site was recorded using an array of five hydrophones. The recordings were then analysed with three methods (see following sections for details):

- Visual and aural inspection of the spectrograms of the recordings;
- Comparison of mean frequency spectra (acoustic fingerprint) within and between sites;
- Statistical analysis of acoustic indices.

Study location

All the recordings were collected in the mid waterholes of the Einasleigh River, Queensland, Australia (approx. S18.07, E143.57; Figure 1). Located in gulf country, Far North Queensland, the Einasleigh River is over 618km long and runs North-West, mostly through arid and semi-arid low open woodland, with mixed level cattle grazing (D.E.H.P., 2016). The region of the river where we conducted surveys is a frontage to Talaroo Station, 31500 ha of destocked pastoral lands, now run as a nature refuge by the Ewamian Aboriginal Corporation (Franklin, Morrison & Wilson, 2017). Climatically, the region is characterised as tropical, with an average annual temperature of 26°C and high annual rainfall from December – March (830mm. weather.mla.com.au), while the rest of the year is very dry. The discharge of the Einasleigh River is greatly dependent on the monsoon and therefore very seasonal, up to $1800 m^3.s^{-1}$ during heavy rain (when combined with the Gilbert River; Gilbert River gauge 917001D; Hydsupp, 2017). Australian ephemeral rivers often contract to isolated river stretches that remain disconnected for up to 10 months each year. These waterholes can be up to multiple km long. Their key characteristic is stagnant water and therefore a more lentic than lotic character. When we conducted the study, the river was an intermittent collection of stagnant isolated pools. They house several soniferous organisms, including at least 3 species of fish from the family Terapontidae, as well as multiple taxa of Hemiptera and Coleoptera (Linke *et al.*, 2018a).

This location was chosen for the study for two main reasons: there are a known variety of soniferous organisms that reside within the river; and it is far enough away from major centres of human population to ensure minimal to null anthropogenic noise. Four waterholes were selected along the river under the following criteria: Pool width > 10m; Pool length > 25m; Depth at centre ~ 1m; and no objects severely impeding placements of our recording frames in transect (see below). Sites were also chosen to be more than 200m apart (Fig. 1).

Experimental design

At each site, an array of five hydrophones was deployed on a 14 m linear transect. Using measuring tape, each hydrophone was separated by 3.5 m from its nearest neighbours. Hydrophones were suspended on frames, 20 cm from the surface to minimise interference from surface reflections, and as a method of controlling for depth-dependent heterogeneity. The frames were made of uPVC pipes (electrical conduit) and assembled using waterproof glue and gaffer tape to reinforce the structures. Five H2a hydrophones (Aquarian Audio, Anacortes, WA, USA) were connected to a single F8 portable recorder (Zoom, Tokyo, Japan) for synchronised recordings, labelled H1-H5. We recorded four times a day for a duration of 45mins; at Dawn (7 am), Midday (12 pm), Dusk (6 pm) and Midnight (12 pm), for a total of 16 x 45 minute recordings. These times were chosen to maximize the diurnal variation of acoustic activity as they are known to be typical times of turn over or maximal diversity (Linke *et al.*, 2018a) while keeping the sampling manageable with such non automated recorders. The recordings were conducted on four different days with stable climatic conditions without extreme conditions such as strong wind or rain. All the recordings were saved as multi-channel in the lossless-format WAV at a sampling rate of 96 kHz and 24 bit. The recordings were later converted to 44.1 kHz to remain within the optimum useable range of the non-scientific hydrophones. Due to technical faults the recordings obtained by H4 were removed from the analysis for this study.

Audio pre-processing and inspection

To optimise the signal to noise ratio (SNR), all the recordings underwent noise reduction in the software Audacity (Audacity Team, 2015, http://audacity. sourceforge.net/). We used the default settings of noise removal using a standard background noise profile (extracted from recordings). This function reduces the intensity of any frequency that is at the average level of the noise profile. We then applied a high-pass filter to all files, set at 0.5kHz with 6dB roll-off per octave to remove interference from wind but retain fish and insect sounds that range between 0.5 and 15 kHz (see Linke et al., 2018a).

An initial aural and visual inspection of recording waveforms and spectrograms was performed using Audacity with window size of 2048 samples, and Hanning window type. This allowed inspection of the most common classes of sounds and their temporal distribution and frequency band. Although sound based species identification is still impossible for most species in freshwater environments due to the limits of scientific knowledge and the lack of sound libraries (Anderson *et al.*, 2008; Desjonquères *et al.*, 2015, 2018; Desjonquères, Gifford & Linke, 2019; Linke *et al.*, 2018a), we had sufficient knowledge to recognise major biological groups signalling in these sites as well as abiotic sounds.

Mean frequency spectra

Differences within and between sites, as well as diurnal variation were assessed with mean frequency spectra. They were calculated with short term Fourrier transforms with a 1024 sample time window. The amplitude value for each equally spaced frequency bins was normalised and then averaged using the arithmetic mean over one minute. The mean frequency spectra were created using the *meanspec()* function in the R package *seewave* (Sueur *et al.*, 2018). To study the between-site spatial and temporal heterogeneity, the amplitude of all four hydrophones was averaged together to build a site profile, so that each chart is the average for the whole site. $10^{th}/90^{th}$ percentile values were overlaid as an indicator of the 45 minute temporal variation. To study the within-site spatial heterogeneity, *meanspec()* was computed independently on each of the four hydrophone channels at each site and averaged over time.

Acoustic indices

Acoustic indices are mathematical functions designed to evaluate some aspects of the acoustic diversity (Sueur *et al.*, 2014). They compute specific features of the spectrum or waveform thought to represent meaningful information about biodiversity (Gage, Towsey & Kasten, 2017). In this study, we employed three acoustic indices: the Acoustic Complexity Index (*ACI),* the Spectral

Entropy (H_f), and the Median of amplitude envelope (*M)*. These three indices were chosen because they measure different aspects of the soundscape, they have been demonstrated to efficiently represent soundscapes and have been used before in freshwater environments (Desjonquères *et al.*, 2015; Linke & Deretic, 2018; Towsey *et al.*, 2018; Buxton *et al.*, 2018b). All three indices were calculated on the whole spectrum in R using the *seewave* package (Sueur et al., 2018). We chose to assess indices over the whole spectrum rather than over any specific frequency band as we were interested in the overall soundscape and not in any given species or taxonomic groups. *ACI* is a measure of spectral complexity – it calculates the average difference of spectral amplitude between time windows (Pieretti, Farina & Morri, 2011). We used *ACI* over the whole recorded spectrum (0-22kHz), and used the default settings in *seewave* (window length = 512 samples, 0% overlap, Hanning type window). H_f is a spectral complexity index. It is analogous to the Shannon Entropy Index from community ecology: instead of species probability of presence, H_f 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. Entropy indices such as H_f are maximised by even spectrum profiles such as white noise while they are minimised by pure tone (Sueur *et al.*, 2008). Accordingly, we observed that the filtered and noise-reduced recordings containing no sounds had H_f close to 1 and recordings with sounds had smaller H_f values. As such, $1 - H_f$ was used, so that the baseline became 0. *M* is a measure of overall intensity of the recording – it calculates the median of the amplitude envelope (Depraetere *et al.*, 2012). The values for H_f and *M* were heavily right-skewed, thus we log-transformed them.

Statistical analysis of acoustic indices

Differences in acoustic index values within and between sites were analysed using three two-way ANOVAs (one for each index) and Tukey's HSD post-hoc tests. *M*, *ACI* and H_f were included as response variable and time of day, site, their interaction (time of the day x site, to estimate the combined effect of site and time of day), and hydrophones (as nested factors within site) were included as explanatory variables to test for temporal variations, and spatial variation between- (sites) and within-sites (hydrophone). We checked for normality and independence of the residuals. Autocorrelation was apparent in *ACI* from consecutive minutes being measured. Based on autocorrelation values, we used every fifth minute of recording for index analysis, as it retained most information while reducing the autocorrelation to acceptable levels (Pieretti *et al.*, 2015). Statistical analyses were performed in the R statistical environment (R Core Team, 2015).

Results

Visual and Aural Inspections of Data using Spectrogram

Of the four time periods, aural and visual inspection of the underwater recordings showed least acoustic activity at dawn, and the majority of acoustic activity had frequencies below 5 kHz. Site differences were observable, but less noticeable than temporal differences. Fish sounds were more common during the day (Fig. 2a), as were geophonic and incidental sounds, including surface splashes, clicks, snaps, wind and gas exchange from plants and sediment (Fig. 2b). Wind was most prevalent during the middle of the day, and dominated recordings below 500 Hz (Fig.2c), but large gusts could cover the entire spectrum. Dusk recordings also showed lower acoustic activity than expected as the insect stridulations did not begin until the middle of the night (Fig. 2d). Site differences were apparent, in total acoustic activity, number of different sounds and frequency range of the sounds. Midnight showed the greatest amplitude of sound of all the time periods with abundant insect stridulations in sites 3 and 4. Fish and incidental sounds (below 5 kHz) continued through this time in all sites. A clear distinction could be observed between sites 1-2 and 3-4 at midnight, depending on insect presence.

Mean Frequency Spectra (between site)

Mean frequency spectra revealed clear differences between times and sites (Fig. 3). Acoustic amplitude was relatively low at dawn except for low frequencies at site 4. There was a peak of acoustic energy at approximately 1.7 kHz at all sites, and additional peaks in site 1 (around 0.2 kHz) and in site 4 (under 0.1 kHz). Variation of amplitude levels at dawn were relatively low except at site 1 and 4. Midday showed overall higher amplitude levels across all sites than dawn, with major peaks being again observed at approximately 1.7kHz and 3 kHz. Low frequency energy (<500 Hz) due to the wind dominated the plots at all sites except site 2. Acoustic energy was low above 5 kHz across all sites except at night in site 3 and 4. All the sites except site 2 had quite large variations in amplitude across the frequency range. Acoustic energy decreased at dusk, with similar patterns to dawn. Major peaks were observed at 1.7 kHz at all sites. Variation of amplitude levels at dusk were relatively low at all sites. Midnight showed the greatest differences between sites, due to presence of insect stridulation (7-10 kHz). Site 3 and 4 have large peaks centred at 8 kHz. The frequency peak of insects was absent at site 2, and barely perceptible at site 1. Overall acoustic energy was greatest at site 3 and 4 and lowest at site 1 and 2. Variation of amplitude levels at midnight were relatively low at all sites.

Mean Frequency Spectra (within-site)

To determine how much sound differed within each site, mean frequency spectra were computed for each hydrophone at each site (Fig. 4). The profiles for all four hydrophones were relatively similar. Site 1 had the most variation between hydrophones and site 2 the lowest. There was some variation between hydrophones in sites 3 and 4: frequencies under 7 kHz were quite variable, and although the peaks at 7-10 kHz caused by insects could be observed in both sites, there was within site variation in amplitude levels, potentially indicating patchy distribution of insects. The differences within sites were less important than the overall differences between sites.

Comparing sites and times with acoustic indices

We found a significant interaction between site and time of day for all the indices (Table 1, Fig. 5). To investigate which pairs of times and sites differed, we performed post-hoc tests. We first investigated the differences between sites within time slots (Table S1) and then the differences between times within sites (Table S2).

Using Tukey's HSD post-hoc tests for pairwise comparisons between sites within time slots revealed 29 significant differences out of the 72 comparisons in total (Table S1, Fig. 5). Most differences were observed at dawn and midnight. The three indices highlighted distinct differences between sites, *ACI* being most different from the other two indices. At dawn, there were significant differences for site pair 2-3 for all indices while site pairs 1-3 and 3-4 were only different for H_f and *M*. At midday, there were significant differences only for *M* between all site pairs except 1-2 and 3-4. At dusk, there were significant differences only for *ACI* between all site pairs except 1-3, and 2-4. At midnight, there were significant differences for all indices between all site pairs except 1-4 for *ACI*, 2-3 and 3-4 for H_f and 1-2 for *M*.

Using Tukey's HSD post-hoc tests for temporal pairwise comparisons within sites revealed 52 significant differences out of the 72 comparisons in total (Table S2, Fig. 5). In site 1, there were significant differences between all time pairs except dawn-midday for *M* and H_f, dawn-dusk for *M* and midday-midnight for *ACI*. In site 2, there were significant differences between all time pairs except dawn-midday for *ACI* and H_f, dawn-dusk for *M*, and dawn-midnight and midday-midnight for *ACI*. In site 3, there were significant differences between all time pairs except dawn-midday and dawn-midnight for *ACI,* midday-midnight for *ACI* and H_f, dawn-dusk for *M* and midday-dusk and dusk-midnight for H_f. In site 4, there were significant differences between all time pairs except dawn-dusk for *ACI* and *M*, and midday-midnight for *ACI* and H_f.

Overall excluding some specific cases, time of day showed consistent differences: dusk and dawn had the lowest index values, while midday had the highest. Midnight revealed greatest difference between sites.

We also found difference within sites with the hydrophones being significantly different for all the acoustic indices (Table 1). The pairwise comparisons revealed few significant differences between hydrophones within sites (Table S3): out of a total of 24 within site comparisons, *ACI* had 4 significant, 9 for H_f and 4 for *M*.

To compare the amount of variance resulting from different factors, we looked at the mean squares of the ANOVA (Table 1). For all three indices, most of the variance was due to time of day, then between-site differences, and within-site differences had the lowest values.

Discussion

We observed distinct spatio-temporal variations within and between sites in this river. Our findings suggest that acoustic patterns of river waterholes are most influenced by diel variation followed by between waterholes variation, and that the lowest source of variation comes from within waterholes. Therefore if the aim is to cover most of the acoustic diversity of a given site in this river, it is most efficient to record from a single hydrophone over multiple times of day.

The highest source of acoustic diversity variation stemmed from diel patterns: fish were most active during the day, and least active at dawn, while insects started calling at dusk, peaking at midnight and finishing at dawn. In a parallel study that investigated the full diurnal acoustic variation in the same river, Linke et al., (2018a), found that as the insects ceased sound production at dawn, the fish began. Indeed such temporal patterns have been observed in other underwater communities (Ruppé *et al.*, 2015). This temporal separation of fish and insects could suggest a temporal partitioning to prevent overlap interference, although frequency overlap between fish and insects are relatively limited.

Our results also revealed significant differences between sites despite their similar characteristics in width, length and depth. Although each waterhole was recorded on a different day, Linke et al., (2018a) revealed that over six days in the same river, the prevalent source of variation was diurnal and that between day variation was relatively low. Moreover we ensured that the meteorological conditions did not differ greatly between days. We therefore believe that differences observed were due to differences in sites, not between days. One main difference between the sites is the presence and intensity of insect chorus between 7 and 12 kHz which lasted most of the night and sometimes through dawn, which is clearly indicated by H_f and M. This difference is mainly due to the chorus of an extremely loud species of the genus *Micronecta* (Sueur, Mackie & Windmill, 2011). Significant differences between sites can be driven by a single species. Our results thus suggest that even in a single river, strong differences can be highlighted by recording at different sites and at different times of the day.

Within site, the overall soundscape was relatively homogenous, despite underwater sounds being limited in how far they can propagate. In our study, the depth of the waterholes varied between 0.5 and 1 m, according to Forrest et al. (1993) this would result in a cut-off frequency for the high-pass properties of shallow water of approximately 0 to 2 kHz. Therefore, even low frequency species such as fish were successfully monitored and in many instances several hydrophones registered the same or similar sounds. Both the mean spectra and the indices indicated that within site differences were relatively small. This suggest that maximising the within-site cover is only of secondary importance to capture a representative sample in a river. This could be due to a relative homogeneity of the micro-habitat or to the wide propagation of sounds within these waterholes. In any case, it would be interesting to investigate further the propagation of sounds in these environments and identify the factors which explain the relatively low impact of within-site variations.

The indices used in this study were chosen for their relative ease of interpretation. They yield a relatively simple single measure of different aspects of a soundscape (amplitude, spectral complexity or spectral variability). The use of acoustic indices is still relatively new. There is therefore a strong need to study their efficacy in various environments and establish evidence-based best practices (Buxton *et al.*, 2018a; Bradfer-Lawrence *et al.*, 2019). Each index describes different attributes of the soundscape, therefore all three indices did not reveal exactly identical results (Dema *et al.*, 2018). For example, while H_f and M reveal the difference between sites 3-4 with insects calling at midnight and sites 1-2 without, *ACI* does not pick up on this difference. Insect stridulations can be very regular temporally and if a chorus is dense enough, it may form a continuous frequency band with little to no amplitude modulation (Ferreira *et al.*, 2018; Desjonquères *et al.*, 2018b). Although *ACI* is designed to ignore such regularities in the spectrogram – similar to continuous anthropogenic noise (Pieretti *et al.*, 2011) – some studies have successfully detected insect choruses using *ACI* (Linke *et al.*, 2018a), this variation in efficiency for *ACI* may be due to the call structure of different insect species. On the other hand, M is an index based on amplitude, it therefore does not differentiate between sounds emitted at different frequencies. Finally, previous studies have found that higher mean H_f values are correlated with greater number of sound types, and that greater H_f indicates less regularity of the acoustic signals (Sueur *et al.*, 2008; Towsey *et al.*, 2014a; Harris, Shears & Radford, 2016). We observed the opposite pattern here, although we did not measure directly sound type richness. Previous studies have shown that low signal-to-noise ratio (SNR), similar to that observed in our study, reduces accuracy and reliability of entropy indices such as H_f; these indices therefore rely upon appropriate filtering to return meaningful results (Depraetere *et al.*, 2012; Parks *et al.*, 2014; Gasc *et al.*, 2015; Desjonquères *et al.*, 2015). Future application in freshwater environments of *Hf* would require to increase the SNR to maximise the efficiency of this index. Index results and interpretation often depend on the ecological questions addressed, the target species and the monitoring approach. Acoustic indices address complementary aspects of a soundscape, we therefore recommend to use them collectively as previously suggested by others (Towsey *et al.*, 2014b; Phillips *et al.*, 2018; Dema *et al.*, 2018).

Overall when monitoring using passive acoustic methods, several considerations should be taken into account to design the spatio-temporal sampling for a study. The main consideration is the aim of the study: is the aim to monitor a specific species, population, or community, estimate diversity, or evaluate ecosystem condition? These aims will result in very different monitoring designs. For example, species and population level studies could maximise spatial coverage and limit temporal coverage by only monitoring during the activity period of the target species. Future research could focus on comparing the species-specific detectability in function of the spatial design of sampling. Species can, for example, vary in how loud and mobile they are, which affects how detectable they are. We expect that high mobility and high signal amplitude species are easier to detect. Spatially explicit capture-recapture (SECR) studies using a hydrophone array similarly to Stevenson et al. (2015) would be the most appropriate method to estimate such detectability most accurately. There are also different analysing tools, including listening, acoustic indices, or automatic detection, with various advantages and issues. On one hand, manual aural and visual inspections can establish a solid ground truth but they are time consuming and may not be a viable option for long-term datasets such as those obtained through PAM. On the other hand, acoustic indices and automatic detections do not require much time to be applied but they still need research and development to be applied widely and interpreted accurately (Bradfer-Lawrence *et al.,* 2019). This is crucial for newly investigated environments such as freshwater ecosystems as most processing methods have been designed for terrestrial environments.

Conclusions

Here we have identified that acoustic variation of underwater environments can be a result of both spatial and temporal factors. This variation exists both within and between local sites of the same river. This means that site selection and recording times requires consideration and knowledge of target species. While temporal variation had previously been identified as an important factor for variability in soundscapes (Desjonquères *et al.*, 2015; Gottesman *et al.*, 2018; Linke *et al.*, 2018a), spatial variation within and between river waterholes had not been investigated. Our results suggest that, if the number of available recording devices is limited, it is crucial to cover various times of the day and several waterholes of an ephemeral river to maximise the capture of acoustic diversity in the soundscape. Monitoring several locations of a single waterhole however appears less essential to capture the overall diversity. In our case, waterholes had low connectivity during most of the year and represented similar habitats. It would be valuable to know if this result holds in more connected reaches or in sites that vary strongly in habitat. It would be especially valuable to see if diel variations are still the strongest source of variation in different environments along an ecological gradient (e.g. altitudinal, eutrophication). We hope our results can be replicated in different rivers and over longer time scales to estimate how generalizable they are to other rivers.

Acknowledgement

We would like to acknowledge the Ewamian people, traditional custodians of the land on which we conducted the research for enabling this project. We especially want to warmly thank Sharon and Lyn Prior, as well as Scott Morrison and the Talaroo ranger team from the Ewamian Aboriginal Corporation for logistical support and friendship. We thank Vanessa Reis, Joe McMahon and Michael Towsey who were involved in the fieldwork. We thank the three anonymous reviewers for their valuable input on the manuscript. SL was supported by ARC DECRA DE130100565. CD was supported by a ENS PhD grant.

Data availability statement

The data used for this project will be made available on Github upon acceptance.

Conflict of interest

The authors have no conflict of interest to declare.

References

Aiken R.B. (1982). Shallow-water propagation of frequencies in aquatic insect sounds. *Canadian Journal of Zoology* **60**, 3459–3461. https://doi.org/10.1139/z82-436

Anderson K.A., Rountree R.A. & Juanes F. (2008). Soniferous Fishes in the Hudson River. *Transactions of the American Fisheries Society* **137**, 616–626. https://doi.org/10.1577/T05-220.1

Bohnenstiehl D.R., Lillis A. & Eggleston D.B. (2016). The Curious Acoustic Behavior of Estuarine Snapping Shrimp: Temporal Patterns of Snapping Shrimp Sound in Sub-Tidal Oyster Reef Habitat. *PloS one* **11**

Bradfer-Lawrence T., Gardner N., Bunnefeld L., Bunnefeld N., Willis S.G. & Dent D.H. (2019). Guidelines for the use of acoustic indices in environmental research. *Methods in Ecology and Evolution*, 2041–210X.13254. https://doi.org/10.1111/2041-210X.13254

Buxton R.T., Agnihotri S., Robin V.V., Goel A. & Balakrishnan R. (2018a). Acoustic indices as rapid indicators of avian diversity in different land-use types in an Indian biodiversity hotspot. *Journal of Ecoacoustics* **2**, GWPZVD. https://doi.org/10.22261/JEA.GWPZVD

Buxton R.T., McKenna M.F., Clapp M., Meyer E., Stabenau E., Angeloni L.M., *et al.* (2018b). Efficacy of extracting indices from large-scale acoustic recordings to monitor biodiversity: Acoustical Monitoring. *Conservation Biology* **32**, 1174–1184. https://doi.org/10.1111/cobi.13119

D.E.H.P. (2016). Einasleigh River drainage sub-basin — facts and maps

Dema T., Towsey M., Sherub S., Sonam J., Kinley K., Truskinger A., *et al.* (2018). Acoustic detection and acoustic habitat characterisation of the critically endangered white-bellied heron (*Ardea insignis*) in Bhutan. *Freshwater Biology*, fwb.13217. https://doi.org/10.1111/fwb.13217

Depraetere M., Pavoine S., Jiguet F., Gasc A., Duvail S. & Sueur J. (2012). Monitoring animal diversity using acoustic indices: Implementation in a temperate woodland. *Ecological Indicators* **13**, 46–54. https://doi.org/10.1016/j.ecolind.2011.05.006

Desjonquères C., Gifford T. & Linke S. (2019). Passive acoustic monitoring as a potential tool to survey animal and ecosystem processes in freshwater environments. *Freshwater Biology*. 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. *Scientific Reports* **8**. https://doi.org/10.1038/s41598-018-31798-4

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

Dufrêne M. & Legendre P. (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological monographs* **67**, 345–366

Farcas A., Thompson P.M. & Merchant N.D. (2016). Underwater noise modelling for environmental impact assessment. *Environmental Impact Assessment Review* **57**, 114–122. https://doi.org/10.1016/j.eiar.2015.11.012

Farina A. & Gage S.H. (2017). *Ecoacoustics: The ecological role of sounds*. John Wiley & Sons.

Forrest T.G., Miller G.L. & Zagar J.R. (1993). Sound propagation in shallow water : implications

for acoustic communication by aquatic animals. *The International Journal of Animal Sound and its Recording* **4**, 259–270
Franklin D.C., Morrison S.C. & Wilson G.W. (2017). A colourful new Australian reaches Talaroo: the Tawny Coster butterfly, Acraea terpsicore. *North Queensland Naturalist* **47**, 10–13
Gage S.H., Towsey M. & Kasten E.P. (2017). Analytical methods in ecoacoustics. *Ecoacoustics. The Ecological Role of Sounds, edited by Farina A. and Gage SH Oxford, UK: Wiley*, 273–296
Gannon D.P. (2008). Passive acoustic techniques in fisheries science: a review and prospectus. *Transactions of the American Fisheries Society* **137**, 638–656
Gasc A., Pavoine S., Lellouch L., Grandcolas P. & Sueur J. (2015). Acoustic indices for biodiversity assessments: Analyses of bias based on simulated bird assemblages and recommendations for field surveys. *Biological Conservation* **191**, 306–312. https://doi.org/10.1016/j.biocon.2015.06.018
Gasc A., Sueur J., Pavoine S., Pellens R. & Grandcolas P. (2013). Biodiversity Sampling Using a Global Acoustic Approach: Contrasting Sites with Microendemics in New Caledonia. *PLoS ONE* **8**, e65311. https://doi.org/10.1371/journal.pone.0065311
Goodman K.J., Parker S.M., Edmonds J.W. & Zeglin L.H. (2015). Expanding the scale of aquatic sciences: the role of the National Ecological Observatory Network (NEON). *Freshwater Science* **34**, 377–385. https://doi.org/10.1086/679459
Gottesman B.L., Francomano D., Zhao Z., Bellisario K., Ghadiri M., Broadhead T., *et al.* (2018). Acoustic monitoring reveals diversity and surprising dynamics in tropical freshwater soundscapes. *Freshwater Biology*. https://doi.org/10.1111/fwb.13096
Harris S.A., Shears N.T. & Radford C.A. (2016). Ecoacoustic indices as proxies for biodiversity on temperate reefs. *Methods in Ecology and Evolution* **7**, 713–724
Hydsupp (2017). Autonomous River and Catchment Data
Indraswari K., Bower D.S., Tucker D., Schwarzkopf L., Towsey M. & Roe P. (2018). Assessing the value of acoustic indices to distinguish species and quantify activity: A case study using frogs. *Freshwater Biology*, fwb.13222. https://doi.org/10.1111/fwb.13222
Linke S., Decker E., Gifford T. & Desjonquères C. (2018a). Diurnal variation in freshwater ecoacoustics: Implications for site-level sampling design. *Freshwater Biology*. https://doi.org/10.1111/fwb.13227
Linke S. & Deretic J.-A. (2018). Ecoacoustics can detect ecosystem responses to environmental water allocations. *Freshwater Biology*
Linke S., Gifford T., Desjonquères C., Tonolla D., Aubin T., Barclay L., *et al.* (2018b). Freshwater ecoacoustics as a tool for continuous ecosystem monitoring. *Frontiers in Ecology and the Environment* **16**, 231–238. https://doi.org/10.1002/fee.1779
Ovaskainen O. & Soininen J. (2011). Making more out of sparse data: hierarchical modeling of species communities. *Ecology* **92**, 289–295. https://doi.org/10.1890/10-1251.1
Parks S.E., Miksis-Olds J.L. & Denes S.L. (2014). Assessing marine ecosystem acoustic diversity across ocean basins. *Ecological Informatics* **21**, 81–88. https://doi.org/10.1016/j.ecoinf.2013.11.003
Phillips Y.F., Towsey M. & Roe P. (2018). Revealing the ecological content of long-duration audio-recordings of the environment through clustering and visualisation. *PLOS ONE* **13**, e0193345. https://doi.org/10.1371/journal.pone.0193345
Pidgeon B. (2003). A review of options for monitoring freshwater fish biodiversity in the Darwin Harbour catchment. *Report for the Water Monitoring Branch, DIPE, Palmerston, 25p*
Pieretti N., Duarte M.H.L., Sousa-Lima R.S., Rodrigues M., Young R.J. & Farina A. (2015). Determining Temporal Sampling Schemes for Passive Acoustic Studies in Different Tropical Ecosystems. *Tropical Conservation Science* **8**, 215–234. https://doi.org/10.1177/194008291500800117

Pieretti N., Farina A. & Morri D. (2011). A new methodology to infer the singing activity of an avian community: The Acoustic Complexity Index (ACI). *Ecological Indicators* **11**, 868–873. https://doi.org/10.1016/j.ecolind.2010.11.005

R Core Team (2015). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Risch D., Castellote M., Clark C.W., Davis G.E., Dugan P.J., Hodge L.E., *et al.* (2014). Seasonal migrations of North Atlantic minke whales: novel insights from large-scale passive acoustic monitoring networks. *Movement ecology* **2**, 1

Ruppé L., Clément G., Herrel A., Ballesta L., Décamps T., Kéver L., *et al.* (2015). Environmental constraints drive the partitioning of the soundscape in fishes. *Proceedings of the National Academy of Sciences* **112**, 6092–6097. https://doi.org/10.1073/pnas.1424667112

Stevenson B.C., Borchers D.L., Altwegg R., Swift R.J., Gillespie D.M. & Measey G.J. (2015). A general framework for animal density estimation from acoustic detections across a fixed microphone array. *Methods in Ecology and Evolution* **6**, 38–48. https://doi.org/10.1111/2041-210X.12291

Sueur J., Aubin T., Simonis C., Lellouch L., Brown E.C., Depraetere M., *et al.* (2018). Package 'seewave'

Sueur J., Farina A., Gasc A., Pieretti N. & Pavoine S. (2014). Acoustic Indices for Biodiversity Assessment and Landscape Investigation. *Acta Acustica united with Acustica* **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

Towsey M., Parsons S. & Sueur J. (2014a). Ecology and acoustics at a large scale. *Ecological Informatics* **21**, 1–3. https://doi.org/10.1016/j.ecoinf.2014.02.002

Towsey M., Zhang L., Cottman-Fields M., Wimmer J., Zhang J. & Roe P. (2014b). Visualization of long-duration acoustic recordings of the environment. *Procedia Computer Science* **29**, 703–712. https://doi.org/10.1016/j.procs.2014.05.063

Towsey M., Znidersic E., Broken-Brow J., Indraswari K., Watson D.M., Phillips Y., *et al.* (2018). Long-duration, false-colour spectrograms for detecting species in large audio data-sets. *Journal of Ecoacoustics* **2**, IUSWUI. https://doi.org/10.22261/JEA.IUSWUI

Villanueva-Rivera L.J., Pijanowski B.C., Doucette J. & Pekin B. (2011). A primer of acoustic analysis for landscape ecologists. *Landscape Ecology* **26**, 1233–1246. https://doi.org/10.1007/s10980-011-9636-9

Tables

Table 1: Effect of time of day (temporal variation), site (between-site spatial variation), their interaction, and hydrophone (within-site spatial variation) on acoustic diversity. Results of an ANOVA for *M*, *ACI* and H_f with time, site, their interaction and hydrophone as explanatory variables. *df*: degrees of freedom, *MS*: mean squares, *F*: F value, *Pr*: P-value.

	ACI				H_f				M			
	df	**MS**	**F**	**Pr(>F)**	**df**	**MS**	**F**	**Pr(>F)**	**df**	**MS**	**F**	**Pr(>F)**
Time	3	1760	54.37	<< 0.001	3	6.25	184.25	<< 0.001	3	11.17	591.06	<< 0.001
Site	3	1466	45.29	<< 0.001	3	1.14	33.70	<< 0.001	3	2.24	118.80	<< 0.001

Site:Time	9	524	16.20	<< 0.001	9	0.73	21.65	<< 0.001	9	2.47	130.78	<< 0.001
Hydrophone	12	232	7.16	<< 0.001	12	0.49	14.52	<< 0.001	12	0.13	6.65	<< 0.001
Residuals	560	32			560	0.03			560	0.02		

Figures

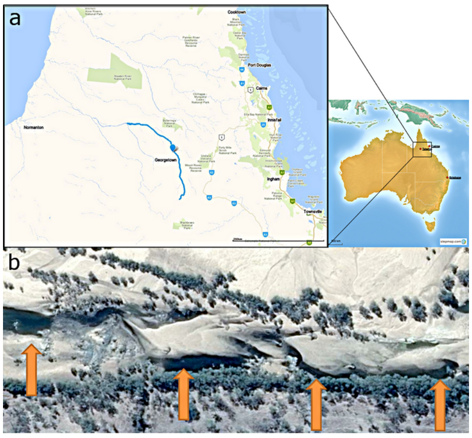

Figure 1: Study location. (a) Map of north Queensland, showing the location of the Einasleigh River. Marker shows Talaroo Station. Image from whereis.com (left) and stepmap.com (right). (b) Birds eye view of the Einasleigh River. Arrows show site 1 (left) to site 4 (right).

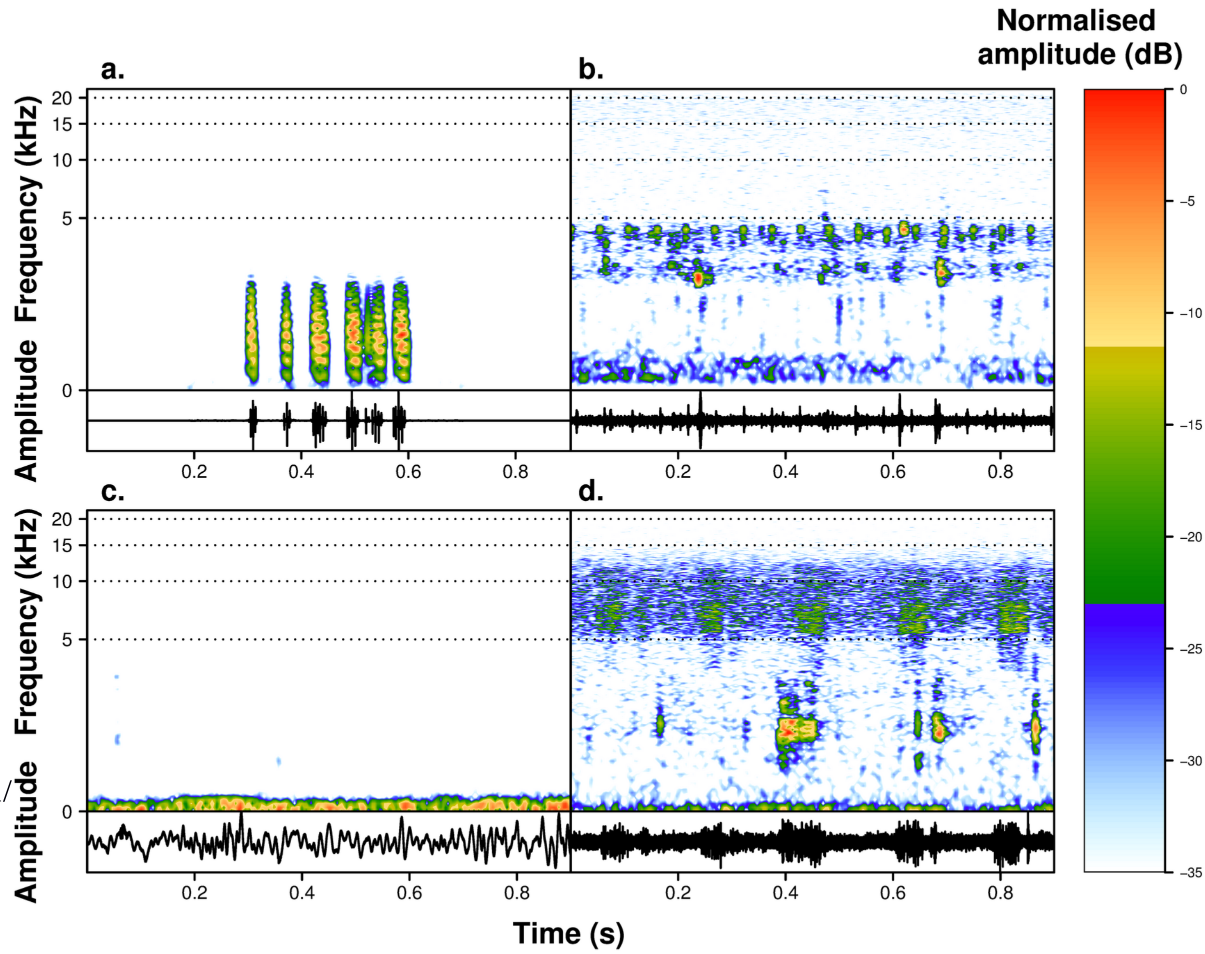

Figure 2: Spectrograms showing the observed acoustic diversity of the four sites. Spectrograms obtain with *seewave*, with a Hanning window length of 1024 samples and 80% of overlap between windows. (a) sound of a fish; (b) ticking and gurgling sounds resulting from gas exchanges; (c) wind sound; (d) ticking sounds linked to gas exchanges in the low frequency and continuous insect chorus sound between 5 to 15 kHz.

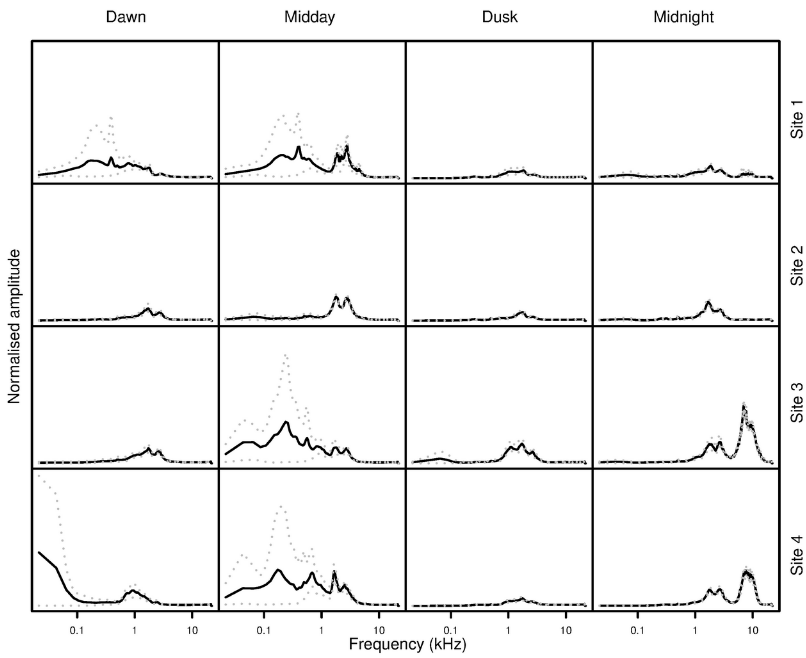

Figure 3: Average frequency spectra for each site/time. Frequency (x axis) is in log scale. Amplitude is presented as a value relative to the maximum amplitude recorded. Full black lines are the averages while grey dotted lines show 10 and 90% percentiles.

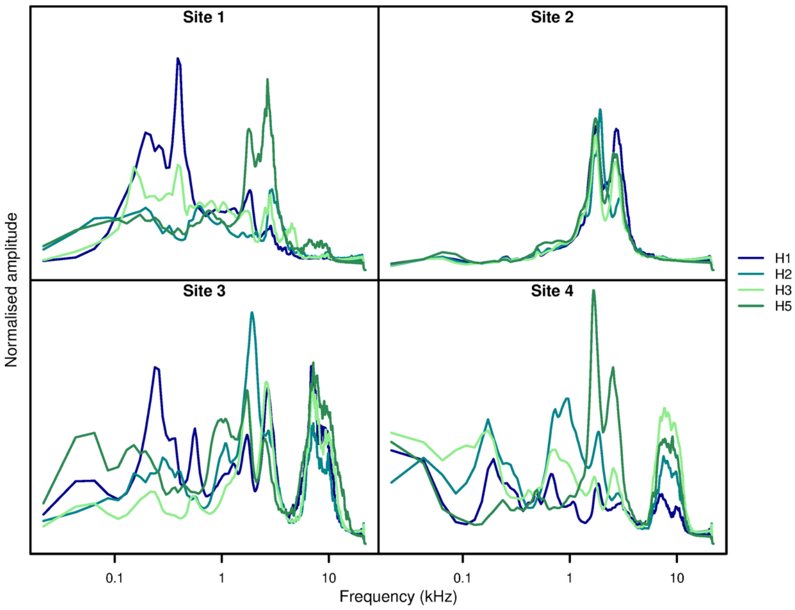

Figure 4: Mean frequency spectra for hydrophone 1-3 and 5 at each site.

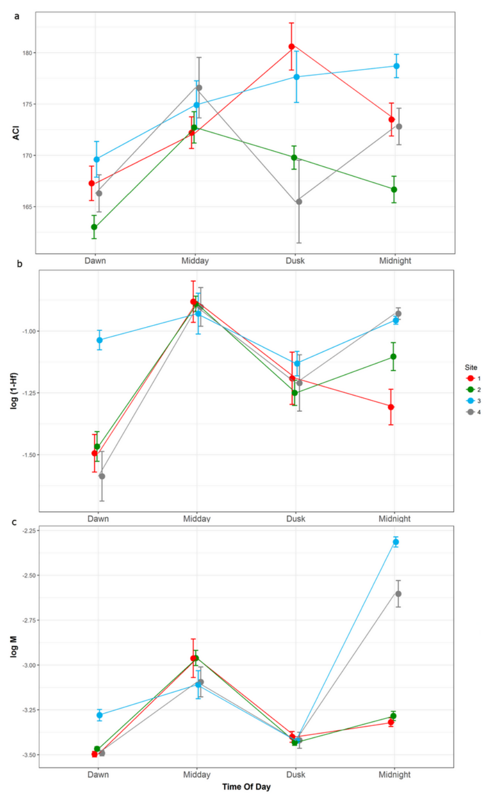

Figure 5: Interaction plot from the two-way ANOVA for the three acoustic indices. Error bars show the 95% confidence intervals.