**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

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17 **Abstract**

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

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

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

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

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

37

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

40 **Introduction**

41 Traditional monitoring of freshwater ecological communities has major limitations: animals are
42 subject to injuries or mortality with methods such as netting, trapping and electrofishing (Pidgeon,
43 2003); often, spatial and temporal variation cannot be obtained without many devoted hours of
44 study (Goodman *et al.*, 2015); and uncommon or rare species are hard to account for (Dufrêne &
45 Legendre, 1997; Ovaskainen & Soininen, 2011). Additionally, in low visibility areas, such as turbid
46 rivers, visual inspections are often impracticable. One alternative approach that mitigates these
47 issues is to monitor the sounds in the environment (Linke *et al.*, 2018b).

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

65 Sound is far less attenuated in water than air. Thus some marine mammals can be recorded
66 from several km away (Risch *et al.*, 2014). However marine mammals produce extremely high
67 amplitude, and low frequency sounds in the open ocean . Sounds of freshwater organisms (such as
68 insects or fish) have lower amplitudes. An important proportion of freshwater environments, such
69 as small ponds and streams, are shallow. In such environments, sound propagation is complex due
70 to the reflection of sound at the bottom and surface of the water (Farcas, Thompson & Merchant,
71 2016). Sound propagation in freshwater environments may be even more complex due to the
72 presence of vegetation, and to the diversity of sediment nature (e.g. soft and organic, sandy or
73 rocky). The few studies on sound propagation in freshwater environments have shown that sound
74 attenuates over less than a meter (Aiken, 1982) and that shallow waters act as high-pass filters, with
75 the cut-off frequency getting lower as the water column gets deeper, according to the theory of
76 waveguides (Forrest, Miller & Zagar, 1993).

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

84 Only a few studies address spatio-temporal variation in freshwaters and their consequences
85 for PAM (Linke *et al.*, 2018b a; Gottesman *et al.*, 2018). Therefore, there is a need to investigate
86 how to design appropriate sampling protocols to account for the various sources of heterogeneity.
87 Here we investigate the extent of spatio-temporal variations in a freshwater environment. Using
88 PAM in four separated waterholes of an ephemeral river, our specific aims were to: 1. determine the
89 extent of variation of underwater sound between nearby waterholes of the same river; 2. determine

90 the extent of spatial variation of underwater sound within river waterholes; 3. estimate diurnal
91 variation in underwater sounds. This variation has already been estimated in other studies
92 (Desjonquères *et al.*, 2015; Linke *et al.*, 2018a) but was not previously compared to spatial
93 variation; 4. compare variation due to spatial and temporal factors observed in underwater sounds;
94 5. interpret how these variations may affect acoustic assessments conducted with different sampling
95 regimes and methods of analyses. We conclude by suggesting best practices and future research
96 necessary to standardise PAM in freshwater environments. Although to estimate the exact sampling
97 effort required, we would need to measure species-specific detection probability, this objective is
98 beyond the scope of our study. In this study, we undertake the first step to standardising protocols:
99 test whether there is significant spatio-temporal sources of variation and compare the relative
100 contribution of different sources of variability.

101

102 **Material and methods**

103 *Overview*

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

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

110

111 *Study location*

112 All the recordings were collected in the mid waterholes of the Einasleigh River, Queensland,
113 Australia (approx. S18.07, E143.57; Figure 1). Located in gulf country, Far North Queensland, the
114 Einasleigh River is over 618km long and runs North-West, mostly through arid and semi-arid low

115 open woodland, with mixed level cattle grazing (D.E.H.P., 2016). The region of the river where we
116 conducted surveys is a frontage to Talaroo Station, 31500 ha of destocked pastoral lands, now run
117 as a nature refuge by the Ewamian Aboriginal Corporation (Franklin, Morrison & Wilson, 2017).
118 Climatically, the region is characterised as tropical, with an average annual temperature of 26°C
119 and high annual rainfall from December – March (830mm. weather.mla.com.au), while the rest of
120 the year is very dry. The discharge of the Einasleigh River is greatly dependent on the monsoon and
121 therefore very seasonal, up to 1800m³.s⁻¹ during heavy rain (when combined with the Gilbert River;
122 Gilbert River gauge 917001D; Hydsupp, 2017). Australian ephemeral rivers often contract to
123 isolated river stretches that remain disconnected for up to 10 months each year. These waterholes
124 can be up to multiple km long. Their key characteristic is stagnant water and therefore a more lentic
125 than lotic character. When we conducted the study, the river was an intermittent collection of
126 stagnant isolated pools. They house several soniferous organisms, including at least 3 species of fish
127 from the family Terapontidae, as well as multiple taxa of Hemiptera and Coleoptera (Linke *et al.*,
128 2018a).

129 This location was chosen for the study for two main reasons: there are a known variety of
130 soniferous organisms that reside within the river; and it is far enough away from major centres of
131 human population to ensure minimal to null anthropogenic noise. Four waterholes were selected
132 along the river under the following criteria: Pool width > 10m; Pool length > 25m; Depth at centre
133 ~ 1m; and no objects severely impeding placements of our recording frames in transect (see below).
134 Sites were also chosen to be more than 200m apart (Fig. 1).

135

136 *Experimental design*

137 At each site, an array of five hydrophones was deployed on a 14 m linear transect. Using measuring
138 tape, each hydrophone was separated by 3.5 m from its nearest neighbours. Hydrophones were
139 suspended on frames, 20 cm from the surface to minimise interference from surface reflections, and

140 as a method of controlling for depth-dependent heterogeneity. The frames were made of uPVC
141 pipes (electrical conduit) and assembled using waterproof glue and gaffer tape to reinforce the
142 structures. Five H2a hydrophones (Aquarian Audio, Anacortes, WA, USA) were connected to a
143 single F8 portable recorder (Zoom, Tokyo, Japan) for synchronised recordings, labelled H1-H5. We
144 recorded four times a day for a duration of 45mins; at Dawn (7 am), Midday (12 pm), Dusk (6 pm)
145 and Midnight (12 pm), for a total of 16 x 45 minute recordings. These times were chosen to
146 maximize the diurnal variation of acoustic activity as they are known to be typical times of turn
147 over or maximal diversity (Linke *et al.*, 2018a) while keeping the sampling manageable with such
148 non automated recorders. The recordings were conducted on four different days with stable climatic
149 conditions without extreme conditions such as strong wind or rain. All the recordings were saved as
150 multi-channel in the lossless-format WAV at a sampling rate of 96 kHz and 24 bit. The recordings
151 were later converted to 44.1 kHz to remain within the optimum useable range of the non-scientific
152 hydrophones. Due to technical faults the recordings obtained by H4 were removed from the analysis
153 for this study.

154

155 *Audio pre-processing and inspection*

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

163 An initial aural and visual inspection of recording waveforms and spectrograms was
164 performed using Audacity with window size of 2048 samples, and Hanning window type. This

165 allowed inspection of the most common classes of sounds and their temporal distribution and
166 frequency band. Although sound based species identification is still impossible for most species in
167 freshwater environments due to the limits of scientific knowledge and the lack of sound libraries
168 (Anderson *et al.*, 2008; Desjonquères *et al.*, 2015, 2018; Desjonquères, Gifford & Linke, 2019;
169 Linke *et al.*, 2018a), we had sufficient knowledge to recognise major biological groups signalling in
170 these sites as well as abiotic sounds.

171

172

173 *Mean frequency spectra*

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

184

185 *Acoustic indices*

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

190 Entropy (H_f), and the Median of amplitude envelope (M). These three indices were chosen because
191 they measure different aspects of the soundscape, they have been demonstrated to efficiently
192 represent soundscapes and have been used before in freshwater environments (Desjonquères *et al.*,
193 2015; Linke & Deretic, 2018; Towsey *et al.*, 2018; Buxton *et al.*, 2018b). All three indices were
194 calculated on the whole spectrum in R using the *seewave* package (Sueur *et al.*, 2018). We chose to
195 assess indices over the whole spectrum rather than over any specific frequency band as we were
196 interested in the overall soundscape and not in any given species or taxonomic groups. *ACI* is a
197 measure of spectral complexity – it calculates the average difference of spectral amplitude between
198 time windows (Pieretti, Farina & Morri, 2011). We used *ACI* over the whole recorded spectrum (0-
199 22kHz), and used the default settings in *seewave* (window length = 512 samples, 0% overlap,
200 Hanning type window). H_f is a spectral complexity index. It is analogous to the Shannon Entropy
201 Index from community ecology: instead of species probability of presence, H_f uses the amplitude of
202 each frequency bin in the mean spectrum (Sueur *et al.*, 2008). This index thus yields a measure of
203 the evenness of the probability mass function. Entropy indices such as H_f are maximised by even
204 spectrum profiles such as white noise while they are minimised by pure tone (Sueur *et al.*, 2008).
205 Accordingly, we observed that the filtered and noise-reduced recordings containing no sounds had
206 H_f close to 1 and recordings with sounds had smaller H_f values. As such, $1 - H_f$ was used, so that the
207 baseline became 0. M is a measure of overall intensity of the recording – it calculates the median of
208 the amplitude envelope (Depraetere *et al.*, 2012). The values for H_f and M were heavily right-
209 skewed, thus we log-transformed them.

210

211 *Statistical analysis of acoustic indices*

212 Differences in acoustic index values within and between sites were analysed using three two-way
213 ANOVAs (one for each index) and Tukey's HSD post-hoc tests. M , *ACI* and H_f were included as
214 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)

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

255

256 *Mean Frequency Spectra (within-site)*

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

264

265 *Comparing sites and times with acoustic indices*

266 We found a significant interaction between site and time of day for all the indices (Table 1, Fig. 5).

267 To investigate which pairs of times and sites differed, we performed post-hoc tests. We first
268 investigated the differences between sites within time slots (Table S1) and then the differences
269 between times within sites (Table S2).

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

279 Using Tukey's HSD post-hoc tests for temporal pairwise comparisons within sites revealed
280 52 significant differences out of the 72 comparisons in total (Table S2, Fig. 5). In site 1, there were
281 significant differences between all time pairs except dawn-midday for *M* and *H_f*, dawn-dusk for *M*
282 and midday-midnight for *ACI*. In site 2, there were significant differences between all time pairs
283 except dawn-midday for *ACI* and *H_f*, dawn-dusk for *M*, and dawn-midnight and midday-midnight
284 for *ACI*. In site 3, there were significant differences between all time pairs except dawn-midday and
285 dawn-midnight for *ACI*, midday-midnight for *ACI* and *H_f*, dawn-dusk for *M* and midday-dusk and
286 dusk-midnight for *H_f*. In site 4, there were significant differences between all time pairs except
287 dawn-dusk for *ACI* and *M*, and midday-midnight for *ACI* and *H_f*.

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

290 difference between sites.

291 We also found difference within sites with the hydrophones being significantly different for
292 all the acoustic indices (Table 1). The pairwise comparisons revealed few significant differences
293 between hydrophones within sites (Table S3): out of a total of 24 within site comparisons, *ACI* had
294 4 significant, 9 for *H_f* and 4 for *M*.

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

298

299 **Discussion**

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

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

313 Our results also revealed significant differences between sites despite their similar
314 characteristics in width, length and depth. Although each waterhole was recorded on a different day,

315 Linke et al., (2018a) revealed that over six days in the same river, the prevalent source of variation
316 was diurnal and that between day variation was relatively low. Moreover we ensured that the
317 meteorological conditions did not differ greatly between days. We therefore believe that differences
318 observed were due to differences in sites, not between days. One main difference between the sites
319 is the presence and intensity of insect chorus between 7 and 12 kHz which lasted most of the night
320 and sometimes through dawn, which is clearly indicated by H_f and M . This difference is mainly due
321 to the chorus of an extremely loud species of the genus *Micronecta* (Sueur, Mackie & Windmill,
322 2011). Significant differences between sites can be driven by a single species. Our results thus
323 suggest that even in a single river, strong differences can be highlighted by recording at different
324 sites and at different times of the day.

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

337 The indices used in this study were chosen for their relative ease of interpretation. They
338 yield a relatively simple single measure of different aspects of a soundscape (amplitude, spectral
339 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 H_f 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).

365

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

385

386 **Conclusions**

387 Here we have identified that acoustic variation of underwater environments can be a result of both
388 spatial and temporal factors. This variation exists both within and between local sites of the same
389 river. This means that site selection and recording times requires consideration and knowledge of

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

402

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411

412 **Data availability statement**

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

414

415 **Conflict of interest**

416 The authors have no conflict of interest to declare.

417

418 **References**

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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).

433 **Figure 2: Spectrograms showing the observed acoustic diversity of the four sites.** Spectrograms
 434 obtain with *seewave*, with a Hanning window length of 1024 samples and 80% of overlap between
 435 windows. (a) sound of a fish; (b) ticking and gurgling sounds resulting from gas exchanges; (c)
 436 wind sound; (d) ticking sounds linked to gas exchanges in the low frequency and continuous insect
 437 chorus sound between 5 to 15 kHz.
 438

440 **Figure 3: Average frequency spectra for each site/time.** Frequency (x axis) is in log scale.
 441 Amplitude is presented as a value relative to the maximum amplitude recorded. Full black lines are
 442 the averages while grey dotted lines show 10 and 90% percentiles.
 443

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

446

447

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