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Monitoring the acoustic activity of an aquatic insect population in relation to temperature, vegetation and noise

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Abstract

1. Acoustic population monitoring is a noninvasive method that can be deployed continuously over long periods of time and at large spatial scales. One of the newly discovered threats acting on biological diversity is anthropogenic noise. High levels of anthropogenic noise occur in aquatic environments, yet their effects on animals living in freshwater habitats have very rarely been investigated.
2. Here, we used acoustic monitoring and automatic detection to assess the acoustic activity of a population of a soniferous freshwater insect.
3. The sounds emitted by the corixid *Micronecta scholtzi* were recorded in a Mediterranean pond with an array of 12 hydrophones. An automatic analysis based on a measure of the amplitude found in the frequency band of *M. scholtzi* was developed to assess the level of acoustic activity. We used functional linear models, accounting for the periodicity of the calling behaviour, to estimate the possible effect of temperature, vegetation and a noise due to an immersed engine.
4. The automatic analysis was validated as an efficient method to measure the acoustic activity. The monitoring revealed a clear 24-hr pattern in the acoustic activity of *M. scholtzi* and three peaks of activity during the morning. Functional linear models revealed negative effects of both temperature and vegetation and showed that an engine noise, played back for 2 hr during the night, elicited an increase in the level of acoustic activity of the population. Moreover, a cross-correlation procedure showed that noise delayed the acoustic activity of the population.
5. Our results suggest that acoustic survey and automatic detection are efficient methods to monitor the acoustic activity of an insect population especially in response to an anthropogenic disturbance.

KEYWORDS
acoustic monitoring, aquatic insect, freshwater habitat, noise pollution, underwater sounds

1 | INTRODUCTION

Current environmental degradations, including climate change, habitat destruction, chemical pollution and anthropogenic noise, impact negatively on natural populations by reducing individual fitness (Groom, 2006). Efficient population monitoring is a key requirement to understand population dynamics induced by these changes and to take appropriate conservation measures. There is an important diversity of methods to monitor populations, from traditional field observation to satellite remote sensing (Le Galliard, Guarini, & Gaill, 2012). Among these census techniques, the sound produced by animals has been suggested as a potential indicator of population status. The use of acoustics started with elementary aural information collected by individual observers (Hutto & Stutzman, 2009). It is now developing quickly with the recent advent of autonomous recorders that can sample audio data regularly over months (Blumstein et al., 2011). Acoustic monitoring therefore appears as a valuable solution to monitor populations of soniferous animals, such as birds, arthropods, amphibians and mammals, with a reasonable workforce and expertise (Laiolo, 2010; Sueur & Farina, 2015). It can be employed to continuously assess the impacts of human-induced disturbances such as climate change (Krause & Farina, 2016) or anthropogenic noise (Barber et al., 2011; Pieretti & Farina, 2013).

Noise pollution due to human activities has been identified as a growing global threat for marine and terrestrial environments (Hildebrand, 2009; McGregor, Horn, Leonard, & Thomsen, 2013). Continuous noises related to transportation and industry and seismic surveys are produced at such pressure levels that they can injure individuals (Popper & Hastings, 2009; Popper et al., 2005), mask communication signals (Fletcher, 2007) or lead to significant modifications in population size, density and demography (Laiolo, 2010). The extent to which noise impacts on natural populations has been mainly assessed in marine and terrestrial environments (Andrew, Howe, & Mercer, 2011; Barber et al., 2011) but rarely in freshwater environments despite a high degree of anthropisation affecting lakes, ponds and rivers (Dudgeon et al., 2006). The anthropisation of freshwater environment is accompanied by the expansion of water-borne sources of noise related to recreational activities, boat transportation and sediment extraction (Bolgan et al., 2016). High-level noise in freshwater environments may have a strong impact on animal populations as observed in marine environments (Tyack & Janik, 2013).

The potential effects of anthropogenic noise are usually assessed at the individual level by estimating either physiological or behavioural changes (Brumm, 2004). These behavioural adaptations have been mainly reported in birds, amphibians and mammals but only rarely in insects although the diversity of their sounds often dominates the acoustic space (Costello & Symes, 2014; Orci, Petróczki, & Barta, 2016; Stanley, Walter, Venkatraman, & Wilkinson, 2016). These adjustments at the specific level are also thought to alter non-soniferous species communities (Solan et al., 2016), by impacting, for example, predation (Simpson et al., 2016), or whole ecosystem and communities (Francis, Kleist, Ortega, & Cruz, 2012) suggesting the occurrence of cascading impacts of noise.

Our goal was to develop and test a monitoring technique to regularly track in space and time the acoustic activity of a population of an aquatic chorusing species. *Micronecta scholtzi* (Corixidae) is a 2–2.5 mm water bug commonly found in temperate freshwater environments. The males of *M. scholtzi* produce an extremely intense stridulation (Reid, Hardie, Mackie, Jackson, & Windmill, 2018; Sueur, Mackie, & Windmill, 2011; Supporting information Figure S1a) that is likely to be involved in intra- and intersexual interactions (King, 1999a). The acoustic communication of *M. scholtzi* and other *Micronecta* species has mainly been studied in laboratory conditions, but field observations showed that thousands of *M. scholtzi* individuals aggregate in dense populations where males form continuous and loud choruses (Jansson, 1977a). This insect is therefore a good candidate for acoustic monitoring in an aquatic environment. We first coined an automatic acoustic process to detect acoustic activity and to estimate its periodicity over days. We used functional data analysis to identify the environmental variables that could influence the spatiotemporal organisation of the population. At last, we used playback of anthropogenic noise to test whether the level and timing of acoustic activity could allow the detection of responses to a change in the environment.

2 | METHODS

2.1 | Study site and data collection

The study site consisted in a Mediterranean pond artificially created in 1992 in the village of Vidauban (Var, France, 43°23′35.0″N 6°27′42.3″E). The pond had an approximate surface of 400 m² with a width ranging from 15 to 22 m (Figure 1). The floor of the pond was made of concrete covered by a layer of soft sediments varying in thickness between 0 and 10 cm. The pond was partly covered by vegetation composed of a single alga species, *Chara globularis*. The spatial limits of the vegetation were visually assessed from aerial pictures taken with a drone (Figure 1), and its growth was estimated every week from the bank of the pond.

The pond was equipped with a network of twelve recording stations each separated by 4.13 ± 0.88 m (mean ± SD, Figure 1) to the nearest neighbour. This distance was chosen to ensure a reasonable

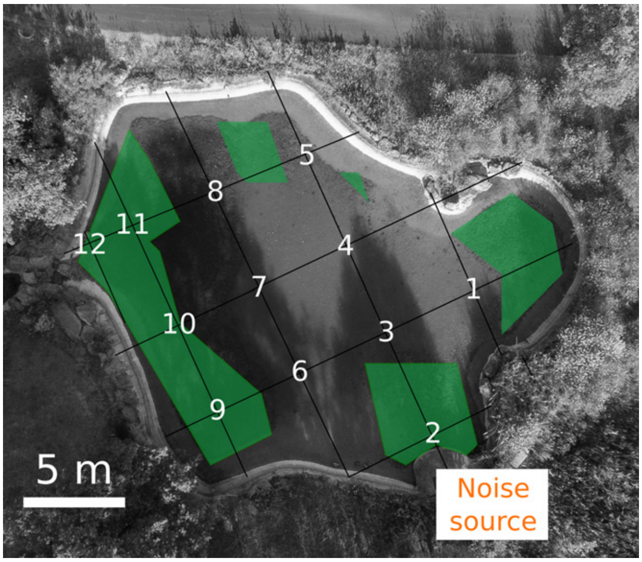

FIGURE 1 Aerial photography showing the location of the recording stations in the pond. The green shaded areas indicate the location of the vegetation, and the numbers indicate the location of each recording station

spatial resolution as well as to avoid recording the same events in neighbouring hydrophones. The water column depth at each recording station was 0.96 ± 0.15 m. A recording station consisted in three units: an autonomous audio recorder (Sound Meter 2+, Wildlife Acoustics, Maynard, MA, USA), a hydrophone (HTI-96, High Tech Inc., Long Beach, MS, USA, flat frequency response between 20 Hz and 40 kHz, sensitivity: −201 dB re: 1 V/μPa) and a thermometer (HOBO Pendant Temperature Data Logger, Onset, Bourne, MA, USA, precision: 0.10°C). The recorder was attached to a tree near the pond. The hydrophone was attached to a rope stretched between a diving weight acting as an anchor and a plastic bottle acting as a buoy. The plastic bottle was filled with polystyrene chips to reduce sound reflections. The hydrophone was maintained at 0.25 m above the sediment, with the piezoelectric sensor directed towards the bottom of the pond. The hydrophones were tested in the laboratory to ensure their frequency responses were identical. The thermometer was attached to the plastic bottle.

The audio recorders were set up to record one minute every 15 min (1 min of recording/14 min of rest, 96 recordings per 24 hr) during 21 days from June 16 to July 7, 2015. The clock of the twelve audio recorders was synchronised with a digital watch so that the twelve stations worked simultaneously. The 24,192 audio files (12 hydrophones × 21 days × 96 recordings per 24 hr) were stored on SD memory cards as uncompressed.wav files at a 44.1 kHz sampling frequency and a 16-bit digitisation depth. The water temperature was recorded at the start of each audio recording.

2.2 | Automatic quantification of acoustic activity level in *M. scholtzi* population

2.2.1 | Measure of the acoustic activity level

Assemblages of acoustically signalling individuals are a widespread collective behaviour among insects and amphibians (Gerhardt & Huber, 2002). Such aggregation form choruses where individual calls are not identifiable. Instead, the multiple calls are merged to a continuous sound over a specific frequency band. In *M. scholtzi*, this frequency band spans from 7 to 12 kHz that cannot be mistaken with any other sound production in this pond as no other species produce sound in this frequency range (Supporting Information Figure S1). A method was designed to automatically quantify the level of acoustic activity of a dense monospecific chorus such as the one formed by *M. scholtzi* based on a measure of the amplitude found in the frequency band of *M. scholtzi*. To measure the amplitude in this frequency band, a short-time Fourier transform (STFT) was applied to each recording. STFT is a standard method to analyse the frequency composition of a digital signal. It consists in segmenting a signal in a succession of regular time windows and in computing the Fourier transform for each window. The Fourier transform decomposes the signal framed by each window into a limited number of Fourier coefficients corresponding to discrete frequencies. Here, the STFT was computed with a window length of 512 samples, a Hanning window type and no window overlap. The Fourier coefficients were not scaled such that the short-time Fourier transform returned a matrix of raw amplitude values which were comparable between every 1-minute recordings. The STFT resulted in an amplitude matrix with frequency bins as rows and time windows as columns. The amplitudes were averaged by row (*i.e.*, over time) to obtain mean values of amplitude per frequency bin over the whole recording, that is, to obtain a mean frequency spectrum. Again, no scaling was applied at this stage so that the amplitude values were raw values. The amplitude values of the mean spectrum for frequencies between 7 and 12 kHz were summed (Supporting Information Figure S2). This amplitude, hereafter referred to as $A_{7\text{-}12}$ or $\log_{10}(A_{7\text{-}12})$ when log-transformed, was computed with the seewave R package (version 2.0.4, Sueur, Aubin, & Simonis, 2008).

2.2.2 | Assessment of the accuracy of the measure

To assess the accuracy of $A_{7\text{-}12}$ to quantify the level of activity of *M. scholtzi* in a recording, an aural assessment was conducted.

The aural assessment was achieved by C.D. who investigated the first 10 s of a subset of recordings, listening to them and visualising their spectrogram with the audio software Audacity (D. Mazzoni, http://audacity.sourceforge.net/). The aural assessment was carried out on the recordings obtained at three different days covering the sampling period (19/06/2015, 27/06/2015 and 04/07/2015) and at six different times of the 24-hr cycle (00:00 am, 02:00 am, 08:00 am, 12:00 am, 04:00 pm and 08:00 pm) resulting in 216 files (12 hydrophones × 3 days × 6 times). The 02:00 am file was selected instead of the file recorded at 04:00 am to avoid recordings in which noise was played back, see Results subsection *Activity of the population in the absence of an anthropogenic noise*. A four-level listening score was designed to classify *M. scholtzi*'s level of activity in each recording (Supporting Information Figure S1c–f): 0: no activity; 1: distinct temporal pattern with a low repetition rate (less than 13 calls in 10 s, corresponding to an estimation of one individual singing continuously; Sueur et al., 2011); 2: distinct temporal pattern with a high repetition rate (more than 13 calls in 10 s, corresponding to more than one individual singing continuously); and 3: temporal pattern not identifiable (corresponding to a high density of individuals singing continuously). A classification tree was used to assess the accuracy of $A_{7\text{-}12}$ (Breiman, Friedman, Stone, & Olshen, 1984). In this classification tree, $A_{7\text{-}12}$ was set as the univariate predictor variable and the listening score as the response variable. The overall accuracy of the tree was used to quantify the correspondence between $A_{7\text{-}12}$ values and the listening score. This analysis was conducted in R using the rpart package (version 2.15.0, Therneau, Atkinson, & Ripley, 2010).

2.2.3 | Control for the effect of background sound level

The background sound level was quantified by measuring $A_{12\text{-}22}$, the summed amplitude values of the mean spectrum for frequencies between 12 and 22 kHz. This frequency band was selected as it was outside Micronecta scholtzi's frequency band and did not contain any other species' sound. The relationship between $A_{7\text{-}12}$ and $A_{12\text{-}22}$

was linear, positive and highly significant (*F*-statistic: 5.3 10^6; adjusted R^2: 0.9956; df: 1, 23498, *p*-value < 0.001, Supporting Information Figure S3). Such a strong relationship indicated that the background noise was entirely determined by the level of activity of *M. scholtzi*. Background sound level was therefore not considered in further analyses.

2.3 | Effect of temperature and vegetation on the acoustic activity of *M. scholtzi* population

The periodicity of the acoustic activity of *M. scholtzi* population was estimated by applying a Fourier analysis on the time series of $\log_{10}(A_{7\text{-}12})$ measured during 7 days, from the 16th to the 21st of June, in the absence of external noise disturbance. The major peak on the amplitude spectrum of the signal was found for a period of 24 hr.

The time (hour) of maximum acoustic activity of *M. scholtzi* was therefore assessed by looking within 24-hr windows. The time of maximum acoustic activity was estimated as the median of all the times of maximum $A_{7\text{-}12}$ values obtained within the 24-hr windows for the 7 days of observations and the 12 recording stations.

Functional data analysis is a statistical procedure using mathematical functions to describe and model smooth variation of a variable. A functional linear model was used to test the importance of the effects of temperature and vegetation (explanatory variables) on the acoustic activity level of *M. scholtzi* estimated with $\log_{10}(A_{7\text{-}12})$ (response variable). The formula of the model was the following:

$$y_{ij} = \mu + \alpha_i + \beta x_{ij} + \varepsilon_{ij}$$

where y_{ij} is $\log_{10}(A_{7\text{-}12})$, i is the index for the vegetation, j is the index for the recording station within a vegetation group, μ is a constant, α_i is the vegetation coefficient, β is the temperature coefficient, x_{ij} is the temperature, and ε_{ij} is the error term.

As the temperature and acoustic activity level were periodic time series, a Fourier basis, that is, a linear combination of sine and cosine functions with specific frequencies, was used to turn them into functional data. The order of the Fourier basis (*i.e.*, the number of sine and cosine in the linear combination) was selected through the observation of the residual part of the function, that is, the pairwise difference between the original signal ($\log_{10}(A_{7\text{-}12})$ or temperature) and the reconstructed signal. The order of the Fourier basis was selected so that the residual part of the model could be considered as random noise and the variance explained by the model was at least of 85%. This comparison led to the selection of a 49 order Fourier basis for both $\log_{10}(A_{7\text{-}12})$ and temperature. The presence or absence of aquatic vegetation at the hydrophone was encoded as a two-level factor (Figure 1). Vegetation was modelled with a constant basis only varying between recording stations as vegetation did not change over the time of the study. The time of day was not added in the model because it was highly correlated with the temperature. Because functional linear models' theoretical null distribution of test statistics is not known, permutation tests were run to test the significance of the model (Ramsay, Hooker, & Graves, 2009). To assess the effect of the vegetation factor, the two levels of the factor were permuted and the explained variance of the initial model was compared to the explained variance of the permuted models. Temperatures were first permuted within each recording station among days to test for an effect of the daily temperature. Temperatures were then similarly permuted over the 7 days of recording to test for an instantaneous effect of temperature. A bootstrap procedure was used to derive confidence intervals for the coefficients of the model. All functional data analyses were conducted with R (R Core Team, 2015) using the *FDA* package (version 2.4.7, Ramsay, Wickham, Graves, & Hooker, 2014).

2.4 | Effect of anthropogenic noise on the acoustic activity of *M. scholtzi* population

The effect of noise on the timing and level of the acoustic activity of *M. scholtzi* population was tested using the engine noise of a water pump. The engine of the pump produced a broadband noise with an irregular spectrum, covering *M. scholtzi*'s frequency band (Supporting Information Figure S1b). This engine could be turned on and off without running the pump such that only noise but no water flow was generated. The engine had never been turned on during the year of the experiment, making this noise an entirely new disturbance to this *M. scholtzi yearly* population.

The response of *M. scholtzi* to noise was estimated on the long term by conducting a 3-week experiment, divided into 3 one-week-long phases. During the first week (16th to 23rd of June), the engine was switched off so that the pond was left undisturbed (pre-treatment). During the second week (23rd to 30th of June), the engine was switched on according to a 24-hr schedule described below (treatment). At last, during the third week (30th of June to 7th of July), the engine was switched off so that the pond was left undisturbed again (post-treatment).

The engine noise and the signal produced by *M. scholtzi* overlapped in time and frequency (Supporting Information Figure S1b) such that it was not possible to disentangle the noise and signal sources. Therefore, the recordings achieved when the engine was turned on were excluded from the analysis. The engine noise was generated for 2 hr each 24-hr cycle that is during 8% of the activity period of *M. scholtzi*, a duration allowing a significant time of exposition to noise while enabling an extrapolation of missing values through a Holt-Winters filtering. As the median time of maximum activity was estimated to occur at 5:15 am (see Results), the engine of the pump was turned on between 3:00 and 5:00 am everyday during the treatment week.

The noise was generated during the part of the activity cycle with an increase in activity, right before the peak of activity so that the activity could either increase or decrease.

2.4.1 | Statistical procedure to test the effect of noise on the acoustic activity level

The null hypothesis (H_0) was that the acoustic activity level was stable during the experiment, and the alternative hypothesis was that the acoustic activity was different in at least one experimental phase (H_1).

To test the effect of the experimental treatment, a functional model similar to the one described above was used, including $\log_{10}(A_{7\text{-}12})$ as the response variable and the temperature, the vegetation and the treatment as explanatory variables. The following model was implemented:

$$y_{ijk} = \mu + \alpha_i + \beta x_{ijk} + \gamma_k + \varepsilon_{ijk},$$

with y_i the $\log_{10}(A_{7\text{-}12})$, i the index for the vegetation with a value of 1 for the absence of vegetation and 2 for the presence of vegetation, j the index for the recording station within a vegetation group (absence or presence), k the index for the experimental phase (1 for pretreatment, 2 for treatment and 3 for post-treatment), μ a constant, α_i the vegetation coefficient, β the temperature coefficient, x_{ijk} the temperature, γ_k the coefficient for the experimental phase and ε_{ijk} the error term.

The order of the Fourier basis was selected so that the residual part of the model could be considered as random noise and the variance explained by the model was at least of 85%. A set of 148 order Fourier basis was used to approximate temperature and $\log_{10}(A_{7\text{-}12})$. A permutation procedure was used to assess the effect of the treatment on the acoustic activity *level*, permuting the three experimental phases. The confidence intervals for the estimated coefficients were derived using a bootstrap procedure.

2.4.2 | Statistical procedure to test the effect of the noise on the acoustic activity timing

The null hypothesis (H_0) was that the timing of activity was the same throughout the duration of the experiment; the alternative hypothesis was that the timing of activity differed in at least one of the experimental phases (H_1).

A cross-correlation procedure was used for each recording station to estimate the time shift between the three pairs of time series obtained pairing the pretreatment, the treatment and the post-treatment. The time series were scaled (mean of 0 and standard deviation of one) over 24 hr. Within each pair of experimental phase, the time shift of maximum correlation between the two time series was assessed. The mean of the time shifts observed at each of recording station was computed and treated with a permutation test including 1000 permutations. The confidence intervals of the time shifts were estimated with a bootstrap procedure.

As shift in acoustic activity timing could also be due to temperature and/or sunrise/sunset time changes, the cross-correlation procedure was also run on the temperature time series and the sunrise/sunset times were compared among the different experimental phases (http://sunrisesunsetmap.com).

3 | RESULTS

3.1 | Validation of the quantification of the level of acoustic activity

When considering all the 24,192 files, the relative amplitude measured between 7 and 12 kHz $A_{7\text{-}12}$ had an average value of $1.02 \cdot 10^6 \pm 1.01 \cdot 10^6$ and ranged from $7.37 \cdot 10^4$ to $1.16 \cdot 10^7$ on a 2^{16} bit scale without unit scale. When considering the 216 files selected for the validation of the quantification of the acoustic activity level, $A_{7\text{-}12}$ fell into the same range of variation with an average value of $8.65 \cdot 10^5 \pm 9.33 \cdot 10^5$.

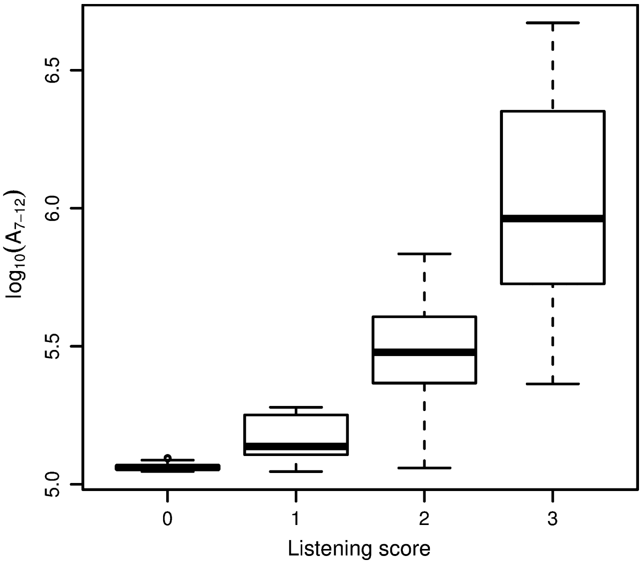

FIGURE 2 Relationship between the listening score and the acoustic activity $\log_{10}(A_{7\text{-}12})$. Box plot showing the 25%, 50% and 75% quartiles. The outliers measured as Q1 – 1.5 * IQD and Q3 + 1.5 * IQD are drawn as points (with Q1, the first quartile; Q3, the third quartile; and IQD, the interquartile distance). The listening score was scaled according to 0: no activity; 1: distinct temporal pattern, with a low repetition rate (less than 13 calls in 10 s, corresponding to an estimation of one individual singing continuously, Sueur et al., 2011); 2: distinct temporal pattern, with a high repetition rate (more than 13 calls in 10 s, corresponding to more than one individual singing continuously); and 3: temporal pattern not identifiable (corresponding to a high density of individuals singing continuously). See Supporting Information Figure S1 for spectrograms and oscillograms illustrating the different levels

The 216 selected files were distributed in the four listening scores, with abundances ranging from 14 for score 1 to 119 for score 3. When no activity was detected, $A_{7\text{-}12}$ had an average value of $1.16 \cdot 10^5 \pm 3.56 \cdot 10^3$ ($n = 29$) indicating a low variation in the background sound level.

The tree revealed 83.8% of overall accuracy in classification of $A_{7\text{-}12}$ into listening scores. According to our expectations, $A_{7\text{-}12}$ increased with the listening score (Figure 2).

3.2 | Activity of the population in the absence of an anthropogenic noise

The acoustic activity of *M. scholtzi* estimated with $\log_{10}(A_{7\text{-}12})$ during the pre-treatment phase showed a regular 24-hr pattern with three peaks of activity, a major peak at 5:15 am and two secondary peaks at 9:00 am and 11:30 pm (Figure 3).

The functional linear model revealed a significant negative relationship between the acoustic activity $\log_{10}(A_{7\text{-}12})$ and vegetation (permutation test: 1000 permutations, p-value < 0.01, Figure 4a,

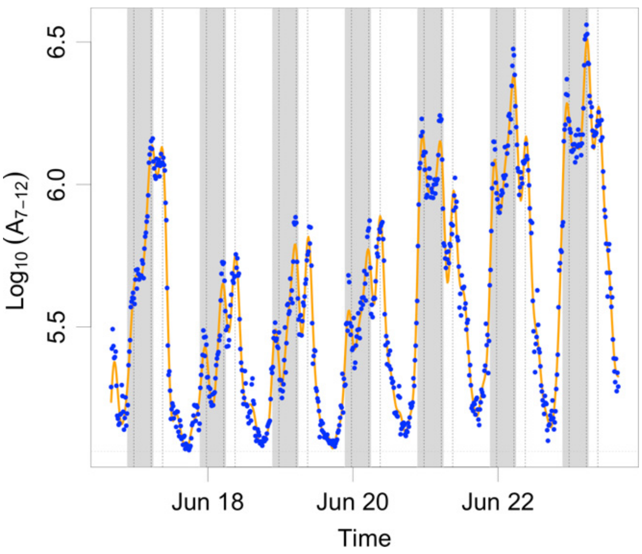

FIGURE 3 Time series of $\log_{10}(A_{7\text{-}12})$ estimated at the recording station 12 for the first experimental phase (pretreatment). The blue points are the values obtained for the recordings obtained every 15 min, and the orange line shows the functional model. The time is expressed as days with shaded areas highlighting the night with sunset at 9:00 pm and sunrise at 6:00 am. The time series shows a periodic pattern with one main peak of activity at 5:15 am and two secondary peaks at 9:00 am and 11:30 pm corresponding to the dotted vertical lines

Table 1). There was no significant relationship between daily temperature and the acoustic activity $\log_{10}(A_{7\text{-}12})$ (permutation test: 1000 permutations, *p*-value = 0.95), indicating that the observed variation of temperature between days did not influence the acoustic activity. There was a negative relationship between the acoustic activity $\log_{10}(A_{7\text{-}12})$ and the instantaneous temperature (permutation test: 1000 permutations, *p*-value < 0.001, Table 1) with similar acoustic activity peaks at temperatures of 26.5 and 28°C, corresponding to the temperatures recorded between 5:00-6:00 am and at 11:00 pm

TABLE 1 Results of the functional linear model for the first experimental phase (pretreatment). The estimate, the 95% lower and upper confidence intervals (CI), the functional version of the *F*-statistic (*F*stat), the number of degrees of freedom (df) and the *p*-values (p-val) are reported for each model term

Term	Estimate	CI_{lower}	CI_{upper}	Fstat	df	p-val
Intercept	5.75	5.66	5.83	[a]	[a]	[a]
Temperature[b]	−0.11	− 0.14	−0.08	0.35	1	**0**
Vegetation[c]	−0.17	−0.42	−0.06	[a]	[a]	[a]

Note. [a]Not shown because of having no meaningful interpretation. [b]Original mean and standard deviation of 27.93 and 1.86°C, respectively. [c]Vegetation was dummy coded with the absence of vegetation as the reference level.

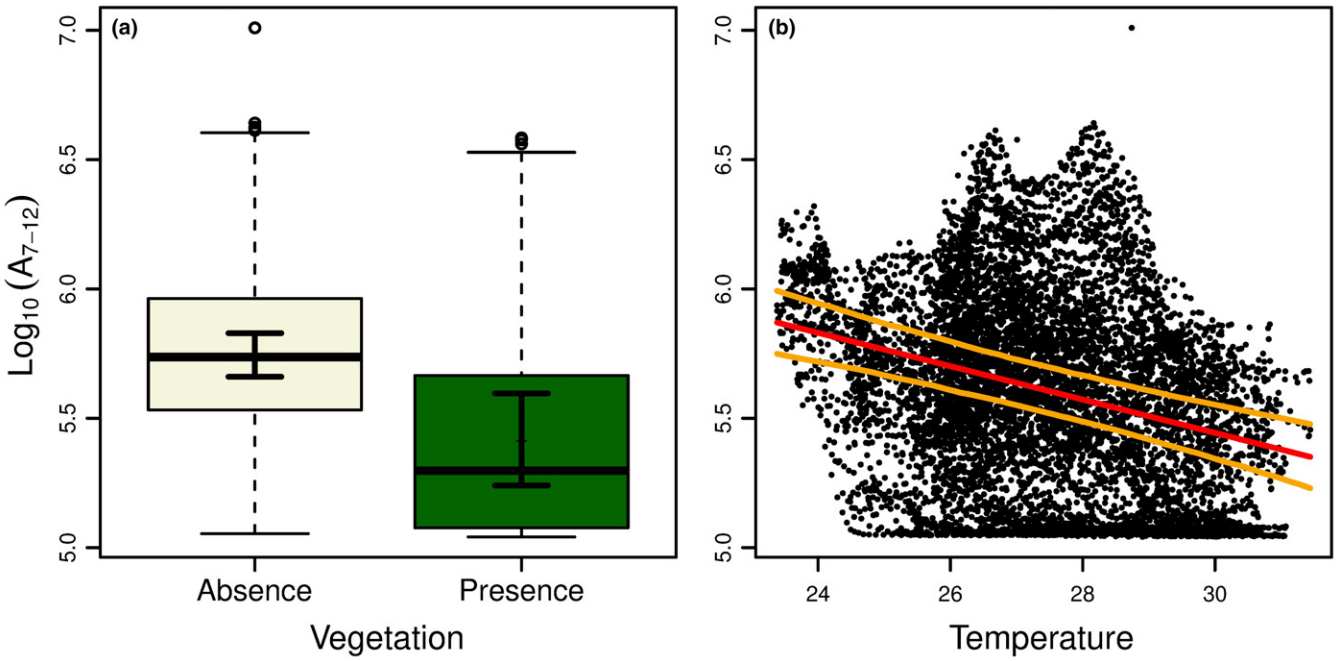

FIGURE 4 Relationship between $\log_{10}(A_{7\text{-}12})$ and vegetation (a), and instantaneous temperature (b). (a). Box plot of $\log_{10}(A_{7\text{-}12})$ according to vegetation showing the 25%, 50% and 75% quartiles and the outliers measured as Q1 – 1.5 * IQD and Q3 + 1.5 * IQD are drawn as points (with Q1, the first quartile; Q3, the third quartile; and IQD, the interquartile distance). The bars in each box show the estimation of the functional model and the 95% confidence interval for these estimations. (b). Scatter plot of $\log_{10}(A_{7\text{-}12})$ in function of instantaneous temperature. The red line shows the fitted functional linear model, and the orange lines show the 95% confidence interval around the fitted values. The lower limit of $\log_{10}(A_{7\text{-}12})$ values (5.03) corresponds to an absence of acoustic activity

TABLE 2 Results of the functional linear model over 3 weeks (pretreatment, treatment and post-treatment). The estimate, the 95% lower and upper confidence intervals (CI), the functional version of the *F*-statistic (Fstat), the number of degrees of freedom (df) and the *p*-values (p-val) are reported for each model term

Term	Estimate	$\mathrm{CI}_{\mathrm{lower}}$	$\mathrm{CI}_{\mathrm{upper}}$	Fstat	df	p-val
Intercept	5.81	5.61	5.89	[a]	[a]	[a]
Temperature[b]	−0.15	−0.26	−0.12	0.65	1	**0.03**
Vegetation[c]	−0.48	−0.59	−0.35	[a]	[a]	[a]
Treatment[d]	0.25	0.18	0.43	[a]	[a]	[a]
Post-treatment[d]	0.28	0.18	0.58	[a]	[a]	[a]

Note. [a]Not shown because of having no meaningful interpretation. [b]Original mean and standard deviation of 29.34 and 2.21°C, respectively. [c]Vegetation was dummy coded with the absence of vegetation as the reference level. [d]Experimental phase was dummy coded with the pretreatment phase as the reference level.

(Figure 4b). Thus, the instantaneous temperature influenced negatively the acoustic activity.

3.3 | Effect of noise on the acoustic activity of *M. scholtzi* population

3.3.1 | Level of acoustic activity

The functional linear model showed estimations of the vegetation and temperature effects similar to the previous model only based on pre-treatment phase (Table 2), with significant negative effects of vegetation (permutation test: 1000 permutations, p-value < 0.01) and instantaneous temperature (permutation test: 1000 permutations, p-value < 0.05). This model also revealed a significant effect of the experimental phase (permutation test, 1000 permutations, *pval* $<$ 0.01, Figure 5) indicating that at least one pair of factor level differed. Checking for pairwise differences, a near significant difference between the pretreatment and treatment phases was found (permutation test: 1000 permutations, Bonferroni corrected *pval* = 0.06, Table 2). The treatment increased the level of activity of *M. scholtzi*. No other pairwise comparison showed a significant relationship (permutation test: 1000 permutations, Bonferroni correction, pretreatment vs. post-treatment: p-value = 0.132, treatment vs. post-treatment: p-value = 1, Figure 5, Table 2).

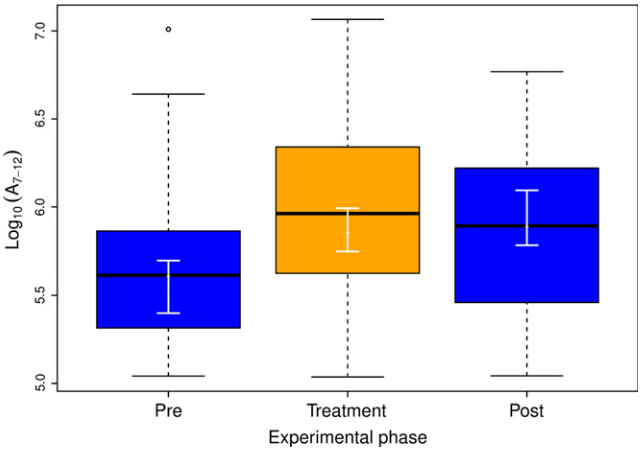

FIGURE 5 Relationship between $\log_{10}(A_{7\text{-}12})$ and experimental phase. Box plot showing the 25%, 50% and 75% quartiles. The outliers are drawn as points measured as Q1 − 1.5 * IQD and Q3 + 1.5 * IQD are (with Q1, the first quartile; Q3, the third quartile; and IQD, the interquartile distance). The white arrow in each box shows the estimation of the functional model for each experimental phase and the 95% confidence interval for these estimations

3.3.2 | Timing of acoustic activity

The time series of $\log_{10}(A_{7\text{-}12})$ showed a significant median positive delay of 10.6 min during the treatment phase (pre-treatment vs. treatment, permutation test: 1000 permutations, p-value < 0.01, Table 3) and of 13.9 min during post-treatment phase (pretreatment vs. post-treatment, permutation test: 1000 permutations, p-value $<$ 0.001, Table 3). No significant changes appeared between the treatment and post-treatment phases (treatment vs. post-treatment, permutation test: 1000 permutations, p-value = 0.168, Table 3).

The temperature was nonsignificantly delayed during the course of the experiment (Table 3). The sunset time shifts were negligible (Table 3). In contrast, sunrise times underwent delays which were not negligible and of the same order of magnitude as $\log_{10}(A_{7\text{-}12})$ for the treatment vs. post-treatment comparison (pre-treatment vs. treatment: 2 min; treatment vs. post-treatment: 3.4 min; and pre-treatment vs. post-treatment: 5.4 min, Table 3).

The delays for sunrise time were lower in the pre-treatment vs. treatment comparison than in treatment vs. post-treatment comparison (2 and 3.4 min, respectively). If the delays of $\log_{10}(A_{7\text{-}12})$ were solely due to the sunrise delay, the delay $\log_{10}(A_{7\text{-}12})$ of pre-treatment vs. treatment would be expected to be smaller than the delay of treatment vs. post-treatment. However, the absence of significant delay for treatment vs. post-treatment indicates that sunrise is not the only source of delay in the level of activity of *M. scholtzi*.

4 | DISCUSSION

Population monitoring is a crucial task for biodiversity assessment and conservation. Acoustic monitoring appears as a potential solution to survey populations of soniferous species. If several methods are in development to acoustically track large animal species such as birds (Bardeli et al., 2010; Furnas & Callas, 2015), frogs (Brandes, Naskrecki, & Figueroa, 2006) or marine mammals (Risch et al., 2014; Zimmer, 2011), almost no attempts have been made to survey insect populations, and, in particular, aquatic insects (Ganchev, Potamitis, & Fakotakis, 2007; Jeliazkov et al., 2016). Here, a simple method based on an array of hydrophones and on the analysis of a single frequency band energy is suited to follow the dynamics of acoustic activity of the waterbug *M. scholtzi*. The method has the great advantages to be noninvasive, time-effective and easy to replicate, three fundamental requirements for population monitoring studies (Blumstein et al., 2011).

TABLE 3 Time shift between pairs of experimental phases for the sunrise, sunset, temperature and acoustic activity. Positive values correspond to delays of the second time series compared to the first (for instance, the sun rises two minutes later on average in the treatment phase than the pretreatment phase). For sunrise and sunset, the mean, minimum and maximum values are reported. For the temperature and $\log_{10}(A_{7\text{-}12})$, the mean values and 95% lower and upper confidence intervals (CI) are reported (p-value *<0.05, **<0.01, ***<0.001)

Comparison	Sunrise time shift (min-max, in minutes)	Sunset time shift (min-max, in minutes)	Temperature time shift (lower, upper CI, in minutes)	$\log_{10}(A_{7\text{-}12})$ time shift (lower, upper CI, in minutes)
Pretreatment vs. treatment	2 (0, 3)	1.1 (0, 2)	0.1 (0, 0.2)	**10.6 (6.1, 16.0)****
Treatment vs. post-treatment	3.4 (0, 7)	– 0.6 (–1, 0)	0.1 (0, 0.2)	2.7 (- 0.7, 6.9)
Pretreatment vs. post-treatment	5.4 (3, 8)	0.6 (0, 2)	0.4 (0, 1.0)	**13.9 (8.2, 20.9)*****

The success of this simple automatic detection is determined by a single condition: there is no other species occupying the same frequency band. The detection method could thus be applied to other species, such as other *Micronecta* species but also other aquatic insects or even amphibians as long as they are the only occupant of a unique frequency band. It is important to note that this method initially allows for activity monitoring. To extend it to population monitoring, the relationship between acoustic activity and population density needs to be modelled. This step could be achieved by coupling acoustic and classical population survey such as standardised netting.

This detection method coupled to efficient time series statistical models allowed to track in space and time the acoustic activity of the population. This method revealed important features on the phenology and ecological preferences of *M. scholtzi*. First, the well-defined day and night pattern of acoustic activity suggests that sound production of *M. scholtzi* is, at least partially, controlled by factors related to solar and/or lunar cycles as observed for most soniferous terrestrial species (Pijanowski, Farina, Gage, Dumyahn, & Krause, 2011). If the method is to be extended to classical population survey, this temporal variation in activity needs to be accounted for.

The ecological significance of *M. scholtzi* has not been estimated, but other species of *Micronecta* have been identified as good indicators of water quality in lakes (Jansson, 1977a). These species produce similar dense choruses for which this method would be efficient. Therefore, this acoustic monitoring could be extended to a range of lakes and ponds, potentially revealing ecological conditions of those environments.

Our acoustic monitoring could highlight a peculiar spatial organisation of the population with a negative relationship between acoustic activity and vegetation. The green alga species, *Chara globularis*, which dominated the vegetation in the pond, could affect by its presence sound propagation such that it could partly affect the intensity of the chorus recorded by the hydrophones. Nevertheless, the marked difference between location with and without vegetation is most likely explained by microhabitat preference of *M. scholtzi* and its affinity for free sediment and open water microhabitats (Jansson, 1977b; CD and JS personal observations). It is interesting that free sediments are associated with favourable conditions of sound propagation with less sound distortion due to obstacles. Such microhabitats may then be chosen also for acoustic properties of the environment allowing an efficient signal transmission, as stipulated by the acoustic habitat hypothesis (Mullet, Farina, & Gage, 2017).

The acoustic activity of *M. scholtzi* population showed an unexpected negative relationship with ambient temperature. Most animal species display a bell-shaped activity response curve to ambient temperature so that an increase in acoustic activity is expected to occur when the temperature rises towards an optimum (Begon, Townsend, John, Colin, & John, 2006). Such an increase has been repeatedly observed in calling rate, carrier frequency and/or amplitude for several ectothermic insects (Sanborn, 2005), including *Micronecta* species (King, 1999b). On the contrary, a decrease in acoustic activity is expected in the right part of the bell-shaped curve where the temperature is too high for an optimal activity. Here, the temperature in the pond had a mean of 29°C and a maximum of 35.1°C, values that were probably relatively high for *M. scholtzi*, a species with a European distribution extending from Denmark to North Africa (Jansson & Seura, 1986). Temperature was naturally correlated with time of the day so that additional experiments in controlled conditions could be run to disentangle the temperature and luminosity effects.

The acoustic activity of the population of *M. scholtzi* varied with experimental phase, suggesting that the engine noise used for the three-week playback experiment did not inhibit but increase the acoustic activity in this pond. This increase in activity was very unexpected, as preliminary monitoring observations revealed that the acoustic activity of the same population in the absence of an engine noise peaked around the 25th of June and then decreased drastically (CD personal observations in 2013) and insects have been shown to decrease their calling behaviour when subjected to traffic noise (Costello & Symes, 2014). Here, the anthropogenic noise had an immediate stimulating effect on the population. In cicadas, a group of terrestrial insects that belong to the same order as waterbugs, the chorusing behaviour of male of *Tibicina haematodes* can be elicited by a noise in the frequency band of the male calling song (Sueur & Aubin, 2002). Here, a roughly similar phenomenon might occur as the noise of the engine and the stridulation of *M. scholtzi* share the same frequency band. Only playback experiments conducted at the individual level and with playbacks of different sounds (different noises and conspecific calls) could confirm this hypothesis. In any case, the increase in acoustic activity linked to the onset of anthropogenic activity could constitute a bias for population survey by increasing the estimated densities.

In addition to a change in the level of acoustic activity of the population, the engine noise also modified the time pattern of acoustic activity. The noise during the treatment phase induced a significant positive delay in the timing of the acoustic activity independently from the effects of a change in the sunrise time. The engine noise stimulated the individual acoustic behaviour resulting in the population being more active and for longer periods. The effects of noise during the treatment phase carried on during the post-treatment phase: Neither the level nor the timing of the acoustic activity recovered the initial values of the pretreatment phase during the post-treatment phase. As *M. scholtzi* was the most important element of the underwater soundscape, such prolonged effects of noise may affect not only the population of *M. scholtzi* but also the complete ecosystem and have consequences on other organisms using sound for communication or orientation following a cascading effect as already revealed in terrestrial and marine communities and ecosystems (Francis et al., 2012; Simpson et al., 2016; Solan et al., 2016). To confirm those potential higher level effects, this experiment would need to be replicated in several ponds and looking at the effect on other species activity.

Ecoacoustics, through a 3-week acoustic monitoring, proved to be a relevant approach to reveal spatio-temporal dynamics of activity in an aquatic insect. Even if, there is still a need for more studies exploring the exact link between acoustic activity and population density, a rigorous and relatively simple protocol was sufficient to identify the spatial and temporal patterns of *M. scholtzi* acoustic activity and to identify effects of noise, temperature and vegetation on this acoustic activity. Such a method could be deployed over several ponds in a monitoring programme designed at a landscape scale, using new open-source, customisable and inexpensive audio recording devices (Whytock & Christie, 2017). Given the current need to monitor effects of global changes, a similar approach could be deployed for other populations such as other aquatic insects, amphibians or fish.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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