MUSEUM NATIONAL D'HISTOIRE NATURELLE

Sciences de la Nature et de l'Homme : évolution et écologie – ED 227

Année 2016

N ° attribué par la bibliothèque
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THESE

Pour obtenir le grade de

DOCTEUR DU MUSEUM NATIONAL D'HISTOIRE NATURELLE

Spécialité : Biologie des populations et écologie

Présentée et soutenue publiquement par

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Le 21 novembre 2016

Ecologie et diversité acoustique des milieux aquatiques : exploration en milieux tempérés

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"Nous ne vivons que pour découvrir la beauté, tout le reste n'est qu'attente"

Khalil Gibran

Waterhole in Talaroo aboriginal reserve (Australia), August 2016

Remerciements

Je voudrais tout d'abord remercier l'Institut SYstématique Evolution et Biodiversité du Muséum national d'Histoire naturelle et l'équipe communications acoustiques du laboratoire NEUROPSY de l'université Paris-Sud pour l'accueil de mon travail de thèse.

J'adresse mes plus chaleureux remerciements à Fanny Rybak et Jérôme Sueur, mes directeurs de thèse pour m'avoir soutenue tout au long de mon doctorat. Ils ont des visions toujours complémentaires mais non contradictoires qui ont enrichi mes travaux. Je les remercie également de m'avoir chaperonnée pour m'éviter une trop grande dispersion (même si j'ai réussi à en garder une bonne dose). Jérôme, je te remercie de m'avoir transmis (autant que possible) ta rigueur méthodologique et conceptuelle ainsi que ta passion pour l'écologie et l'acoustique. Fanny, merci pour nos discussions qui m'ont encouragée à perfectionner mes compétences pédagogiques me permettant ainsi de comprendre et expliquer plus en profondeur mes résultats.

Je souhaite remercier les membres du jury pour avoir accepté d'évaluer mon travail de thèse. Je remercie Régis Céréghino et Almo Farina d'avoir accepté la lourde tâche de rapporteurs pour ce travail. J'exprime également reconnaissance à Emmanuel Castella, Christian Kerbiriou et Nicolas Mathevon, les examinateurs pour leur participation à cette évaluation.

J'adresse ma gratitude aux membres de mon comité de thèse, Emmanuel Castella, Isabelle Le Viol et Eric Parmentier pour leur soutien et nos discussions à propos de mon projet de thèse. Celles-ci m'ont permis d'orienter ce travail et d'avancer dans les directions les plus fructueuses.

Je remercie mes collaborateurs, Avner Bar-Hen, Emmanuel Castella, Marion Depraetere, Amandine Gasc, Toby Guifford, Alexandre Kempf, Isabelle Le Viol, Simon Linke, Diego Llusia, Sandrine Pavoine et Juan Sebastian Ulloa, sans qui cette thèse n'aurait pas été possible.

Durant ma thèse, j'ai eu la chance de travailler dans deux grande institutions et de voyager à l'occasion de conférences et de séminaires, ce qui m'a permis de rencontrer différents chercheurs qui ont influencé ma vision de la recherche et de la science, pour cela, je dois remercier Guillaume Achaz, Avner Bar-Hen, Toby Gifford, Gerlinde Höbel, Simon Linke, Joël Potier, Nicolas Rabet, Tony Robillard, Rafael Rodriguez, Jürgen Schmidl, Michael Towsey et sans doute beaucoup d'autres.

J'ai également effectué trois missions de terrain pendant lesquelles Catherine Fournil, Rémi de l'ONF, Romain Garrouste, Amandine Gasc, David Mac-Raye, Jean-Luc Pessel, Pierre-Alexis Rault, m'ont énormément aidée pour l'installation, le choix des sites et les questions de recherche. Je remercie également mes deux stagiaires, Alexandre Kempf et Julien Stackowicz, pour leur superbe travail sur leurs projets respectifs. Je dois enfin saluer le travail des personnes m'ayant aidé pour la relecture de l'anglais, Peter Naylor (senior) et Bruno Naylor.

Je remercie les sources de financement dont j'ai bénéficié pour leur soutien sans lequel je n'aurais pu effectuer mes expériences de terrain et assister à une conférence en Allemagne, je remercie donc le Labex BCDiv, l'Institut SYstématique, Evolution et Biodiversité ainsi que les amis du muséum.

Je souhaite ensuite remercier mes collègues de bureau et d'étage, Diego, Juan, José, Maram, Monica, Vitor, Noura, Bruno, Pablo, Julien, Amandine, Alexandra, Laurent, Lorène et tant d'autres pour les superbes soirées passées ensemble et les pauses café qui m'ont vraiment permis de survivre à ces quatre années au Muséum. Je remercie aussi les autres amis du muséum, avec qui j'ai pu profiter de la vie étudiante et surtout du BDEM : Corentin (le trésorier verreux), Lucie (la décofougéromaniaque), Laurianne (ma copine), Jérémy (empereur du pop corn), Valentin (la danseuse), Antoine (Eh salut!), Charles (le déserteur), Marc (le chef boisson), Malcolm (le chef boisson, oui y'en a deux!), Donald (mon co-bayeux), Fabien (M. le président), Ninon (l'exemple à suivre), Baptiste (l'homme à tout faire) et tant d'autre! (Désolée pour ceux que j'oublie, je vous aime quand même).

Je remercie aussi mes amis extérieurs au muséum qui m'ont permis sortir du labo, notamment mes amis de l'ENS, Frances, Cyril, Pil, Arno, Jacques, Enzo, Michou, Tristan, Corentin, Bichette et ceux que j'oublie, mes amis de prépa : Quentin, Chloé, Arnaud, Constance, Alexis, Stéphane, Elo, Mehdi, Gobain, Vincent, Jô, Sofia, Anne, Lorraine et les autres! Mes amis d'avant : Jeanne et Aude (mes amies d'enfance), Audrey, Romain, Chouchou, Mathilde, Lucas...

Pour finir, j'aimerais dire un grand merci ma famille, mon père qui m'a fait part de son expérience de recherche et dont j'ai sans doute hérité le sang froid, ma mère qui m'a toujours encouragé, surtout dans mes moments de doute, mon frère et ma soeur qui ont toujours eu confiance en mes capacités. Un grand merci aussi à ma seconde famille, la coloc : Marie (babi), Martin et Pitou (les coloc par adoption), Nelly (bull queen), Ant One (dart champion), T-dawg (mon coloc préféré), LT-dawg (la plus belle allonge), Manion (ma soeur, ma bataille) et enfin Bruno (le plus drôle), la personne qui m'a soutenue tous les jours et toutes les nuits pendant quatre années de vie commune.

Je souhaite à tous une bonne lecture, en espérant que vous y retrouverez un peu de vous !

Contents

List of Figures

List of Tables

Résumé étendu en français

Une grande diversité d'animaux produit des sons pour communiquer, s'orienter, ou lors de la réalisation de divers actes comportementaux, comme la prise de nourriture. Les environnements d'eau douce sont considérés comme les réservoirs d'une importante diversité biologique. Ils abritent potentiellement un nombre significatif d'espèces produisant des sons.

Les sons produits par cette diversité d'espèces peuvent être classés en types de son. Un type de son est une unité sonore définie par un ensemble de paramètres fréquentiels et temporels. Dans un environnement donné, plusieurs individus peuvent produire des sons en même temps constituant des ensembles de sons considérés comme des populations ou communautés acoustiques. Une population acoustique est constituée par un ensemble de sons de même type produits à un temps donné et dans un même lieu. Une communauté acoustique est constituée par un ensemble de sons de types différents produits à un temps donné et dans un même lieu.

L'objectif principal de cette thèse est d'explorer pour la première fois la diversité acoustique présente dans les milieux d'eau douce en climat tempéré, en étudiant la structure des populations et communautés acoustiques tout en explorant les processus pouvant déterminer cette structure.

Cette thèse est structurée en quatre chapitres se proposant de répondre aux questions suivantes: 1/ Quelles sont les sources acoustiques animales en milieux d'eau douce ? 2/ Comment la diversité acoustique des environnements aquatiques d'eau douce est-elle structurée ? 3/ Quels sont les facteurs environnementaux pouvant être reliés à la structure des communautés acoustiques ? 4/ Quels sont les facteurs environnementaux pouvant être reliés à la structure d'une population acoustique et les bruits d'origine anthropique peuvent-il perturber cette structure ?

Chapitre 1 : Qui « chante » sous l'eau ?

Quatre taxons sont connus pour produire des sons en milieu d'eau douce : les amphibiens, les crustacés, les insectes et les poissons à nageoires rayonnées. Les connaissances sur les espèces productrices de sons n'ont jamais été résumées pour tous ces taxons. Le premier chapitre permet de rassembler et d'élargir les connaissances sur la production sonore dans les milieux d'eau douce en France.

Ce chapitre est structuré en deux parties. La première partie synthétise la littérature sur la production sonore dans les milieux d'eau douce. La deuxième partie décrit des enregistrements d'espèces aquatiques effectuées en laboratoire.

La revue approfondie de la littérature révèle qu'au moins 271 espèces produisent des sons sous l'eau dans les environnements d'eau douce en France. Les paramètres fréquentiels et temporels des sons de seulement 35% des espèces été décrits précisément dans la littérature.

19 espèces collectées en milieu naturel sont isolées en aquarium pour être enregistrées en laboratoire dans des conditions contrôlées. Ces espèces ont été sélectionnées d'après la synthèse de la littérature pour représenter des taxons dont les productions sonores ne sont que partiellement voire absolument pas décrites. Parmi les 19 espèces étudiées, huit ont produit des sons au laboratoire, dont deux espèces chez qui l'émission de son n'avait jamais été décrite.

Ainsi, une diversité significative d'organismes produit des sons en milieux d'eau douce. Mais les connaissances sur les sons de ces espèces apparaissent dispersées et incomplètes.

Compléter et rassembler ces connaissances pourrait permettre l'identification et le suivi des espèces par le son, notamment pour des espèces difficiles à observer dans leur milieu naturel.

Chapitre 2 : Exploration de la diversité acoustique subaquatique de trois mares en milieu tempéré

Le deuxième chapitre présente le premier suivi acoustique à long terme dans des mares tempérées.

Grâce à une inspection visuelle et par l'écoute d'une sélection d'enregistrements, un total de 48 types de sons ont été identifiés dans trois mares du nord de la France. Les trois mares comprennent des types de sons différents dont le nombre total varie de huit pour la mare la moins riche à 42 pour la mare la plus riche. Ce nombre total de types de sons estimé par l'échantillonnage est probablement sous-estimé dans l'une des trois mares étudiées. Étonnement, chaque mare montre un motif journalier d'activité particulier : la variation journalière du nombre de types de sons est différente dans chaque mare.

Ce chapitre permet également d'explorer la possibilité de réaliser une évaluation rapide de la biodiversité grâce à des indices de diversité acoustique. Les indices de diversité acoustique sont des fonctions mathématiques conçues pour représenter certains aspects de la biodiversité grâce au son. L'efficacité de six indices de diversité acoustique a été testée. Ces six indices sont l'entropie temporelle H_t, l'entropie spectrale H_f, l'enveloppe de l'énergie M, la richesse acoustique AR, le nombre de pics fréquentiels majeurs NP et l'indice de complexité acoustique ACI. Le test d'efficacité révèle que tous les indices sauf un sont corrélés au nombre de types de son mais ceux-ci sont encore plus corrélés au rapport signal sur bruit. Un second test de corrélation permettant de prendre en compte l'effet du rapport signal sur bruit démontre que la richesse acoustique AR semble être un bon candidat pour estimer le nombre de types de sons.

Des recherches supplémentaires sont nécessaires pour calculer automatiquement le rapport signal sur bruit afin d'appliquer cet indice sur un grand nombre d'enregistrements. Les résultats révèlent que ces trois mares tempérées comportent une large diversité acoustique différant non seulement entre elles mais également au sein des mares. Les sources produisant cette diversité de sons et les facteurs initiant ces différences journalières en types de sons nécessitent une étude plus poussée pour pouvoir associer chaque son à une espèce. Une telle recherche permettrait d'obtenir un aperçu de la biodiversité et de l'écologie des mares grâce au son.

Chapitre 3 : Relier le son et l'écologie

Des études récentes ont révélé que certains motifs et processus écologiques peuvent être examinés grâce aux sons émanant des populations ou communautés. Bien que plusieurs espèces d'eau douce produisent des sons sous l'eau, la structure des assemblages acoustiques des habitats d'eau douce et les processus les façonnant sont mal connus.

L'objectif de ce troisième chapitre est d'étudier le lien entre les variables environnementales et les communautés acoustiques afin de rechercher les facteurs environnementaux façonnant ces assemblages acoustiques. Un suivi acoustique passif est déployé pendant 15 jours dans six bras mort du Rhône. Ces six bras-morts diffèrent par leur connectivité latérale, un paramètre écologique clé régulant la composition des communautés de macro-invertébrés et de poissons.

Un total de 128 types de sons sont enregistrés révélant une diversité acoustique subaquatique importante bien que partiellement connue. Cette diversité, au lieu d'être répartie aléatoirement parmi les six bras-morts, est spécifique à chaque site. Les six communautés acoustiques différentes affichent des signatures acoustiques distinctes identifiées grâce à une analyse multi-variée. Un ensemble de modèles statistiques révèle une relation significative entre la connectivité et la composition des communautés acoustique.

Ces résultats, en accord avec les études précédentes basées sur des échantillonnages classiques, suggèrent que les communautés acoustiques sont façonnées par les interactions avec leur environnement. Ce suivi acoustique soutient donc les avantages potentiels de l'analyse sonore pour décrire et comprendre les motifs écologiques, particulièrement dans les environnements d'eau douce.

Chapitre 4 : Suivi acoustique des effets d'un bruit d'origine humaine sur une population d'insectes aquatiques

Le bruit d'origine humaine est une des nouvelles menaces pesant sur la diversité biologique. Bien que des niveau élevés de bruit anthropique soient présents dans les milieux aquatiques, les effets du bruit sur les animaux d'eau douce n'ont que rarement été examinés. Évaluer les impacts du bruit anthropique et les atténuer requiert un suivi précis des populations. Le suivi de population grâce à l'acoustique est une méthode non-invasive qui peut être déployée de façon continue sur de longues périodes de temps et de larges échelles spatiales.

Dans ce quatrième chapitre, un suivi acoustique est utilisé pour évaluer les effets du bruit sur l'activité acoustique d'une population d'insecte aquatique produisant du son. L'activité acoustique de *Micronecta scholtzi*, une punaise aquatique, a été enregistrée dans une mare méditerranéenne à l'aide d'un réseau de 12 hydrophones. Une analyse spectrale automatique a été développée pour estimer le niveau d'activité de la population d'insecte pendant le cycle journalier et afin d'évaluer les effets du bruit émis par un moteur immergé.

L'activité de la population suit un rythme régulier sur 24 heures avec un niveau d'activité maximum pendant la nuit. Un modèle linéaire fonctionnel contrôlant les effets de la température et de la végétation démontre que la diffusion du bruit durant deux heures pendant la nuit entraîne une augmentation ainsi qu'un retard du niveau d'activité acoustique de la population. Ces changements sont conservés pendant la période suivant la diffusion suggérant un effet à long terme du bruit.

Le bruit d'origine anthropique a des conséquences significatives sur l'activité de cet insecte aquatique. L'invasion des bruits d'origine anthropique implique donc des effets non seulement dans les environnements terrestres et marins mais également dans les milieux d'eau douce.

Conclusion

Cette thèse de doctorat est une première exploration de la complexité, de la structure et du dynamisme des populations et communautés acoustiques. Les différents niveaux structurels révélés dans les divers environnements d'eau douce étudiés suggèrent que les variables environnementales façonnent les caractéristiques sonores des milieux. Avec ce travail, nous révélons une diversité acoustique significative en milieu d'eau douce.

Ces résultats ouvrent de nombreuses perspectives : i) en termes d'exploration, car une importante diversité acoustique reste à découvrir, ii) en termes de recherche fondamentale, car les processus écologiques gouvernant l'assemblage des populations et communautés acoustiques sont à préciser, et enfin iii) en termes d'applications, car l'utilisation de l'acoustique pourrait permettre d'améliorer le suivi en temps réel de ces habitats menacés par les activités de l'homme.

General introduction

Hydrophone

Hydrophone : (hdr-fn)
A device used to detect or monitor sound under water. Hydrophones are often installed or towed in arrays that can be used to pinpoint a sound source or provide sea-floor imaging as part of a sonar system.

[redacted]

Dial a number below to hear a recording

Hydrophone : (hdr-fn)
Un appareil pour détecter or surveiller les sons sous l'eau. Les hydrophones sont souvent installés ou remorqués en réseau pour localiser l'origine des sons ou pour élaborer une carte topographique du fond de la mer.

[redacted]

Composez un numéro et vous entendrez des enregistrements

0	Mulette perlière Fresh Water Pearl Mussels	Un moule peut vivre pour un siècle et devient un adult après 15 ans A mussel lives for up to a century and matures only after 15 years
1	L'Escargot Aquatique Aquatic snails	Les escargots sont très forts et peuvent soulever jusqu'a 10 fois leur poids d'une position verticale Snails are very strong and can lift up to 10 times their own body weight in a vertical position
2	Tench Tench	Les tanches sont souvent repérés parmi les nénuphars par un flot de boules continuel qu'ils laissent échapper pendant qu'ils mangent Tench are often seen feeding among water lily pads by a continuous trail of bubbles
3	Scarabé aquatique Water Beetles	Pour attirer un partenaire les scarabés communiquent en frottant leurs pattes arrières comme les grillons. Chercheurs supposent que la sélection sexuelle puisse etre la raison que la chanson de ces insects atteint des puissances aussi hautes. Beetles communicate for mating by rubbing their hind legs like crickets, Researchers believe that sexual selection could be the reason why the insects' songs reach such high amplitude.
4	Gyrin commun Whirligig Beetle	Incroyablement, la vitesse de ce petit bête, traduit en termes humains, sera environ 290 km par heure Translated into human terms, the speed of this beetle would tbe an amazing 290 KM per hour
5	Notonecte Water Boatman	Notonectes sont parmi les créatures les plus bruyants du monde relatif a son taille. Ils chantent pendant la nuit, et sont souvent entendu a crépuscule. Le bruit est fait en frottant son pénis sur son corps. En asie, il existe une espèce de notonecte qui est mangé comme spécialité gastronomique et qui a un gout de gogonzola! One of the noisiest creatures on earth relative to its size, most vocal at night, best heard at dusk, the noise is made by rubbing its penis on its body. In tropical asia there is a species of water boatman eaten as a delicacy and tastes of gorgonzola cheese
6	Punaise scorpion Water Scorpion	Ces insects ressemblent aux scorpions à cause de leur pinces et leur queue comme fouet. Néanmoins ce queue est en fait un appareil respiratoire très etroit qu'ils utilisent comme tuba. These insects resemble scorpions because of their pincher-like front legs and long whip-like tail piece. Their tail, however, is actually a narrow breathing tube which they can thrust up to the surface like a snorkel.
7	Plantes aquatiques Aquatic Plants	Ce son est des plantes qui photosynthétisent - créant de la nourriture du soleil! The sound is the plants photosynthesising - making food from sunlight!
8	Dytique Great Diving Beetle	Carnivores féroces qui dévorent des têtards ou des petits poissons Fierce carnivores that devour full grown tadpoles and small fish
9	Ecrevisse Freshwater Crayfish	Cet espece est plutot nocturne. Historiquement les villages du vallee du Thames, en angleterre, ont fait des fêtes en l'honneur des écrevisses. Species most active at night, Historically villages in the thames catchment held jolly crayfish parties

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Artwork in an hortillonage near Amiens (Somme, 80), June 2015 (Pictures: Jérôme Sueur)

Bioacoustics: the study of animal acoustic communication

Acoustics and sounds

Acoustics is the scientific discipline studying mechanical waves in various substrates which can be gas, liquid or solid. The term sound usually refers both to the auditory sensation in the ear and the mechanical wave which causes this auditory sensation (Schroeder et al., 2007). Sound is thus often defined as a wave of pressure disturbance propagating through a fluid (liquid or gas) emitted by a vibrating source which induces surrounding fluid particles to vibrate longitudinally (Figure 1). This restricted and anthropocentric definition has been challenged and enlarged following the advent of bioacoustics, the scientific discipline studying the animal acoustic communication systems. A huge biological diversity of mechanisms of sound reception and perception has been discovered. Therefore, in bioacoustics, a sound refers to an acoustic wave propagating in a fluid (liquid or gas) or in a solid (elastic waves). The propagation of elastic wave not only consists in longitudinal waves but also transversal ones, those complex features extend beyond the scope of this thesis. Therefore references to sound will be here mostly related to acoustic waves propagating in fluids except when specified.

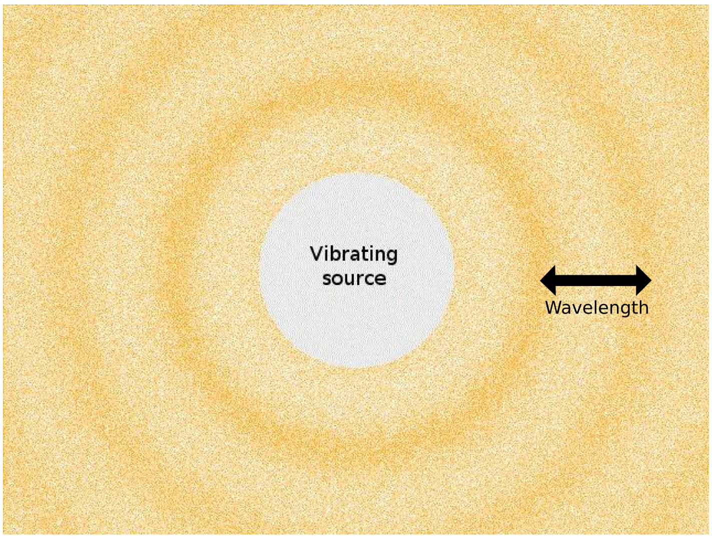

Figure 1 – **Particle motion induced by a spherical acoustic source**. The colour indicates density, or pressure. The white sphere at the center of the figure is the vibrating source. (source: wikimedia, author: Thierry Dugnolle).

Assuming that the source in Figure 1 vibrates at a pure tone, corresponding for example to the musical note A, the motion of one particle in Figure 1 around its equilibrium point can be represented as a sine wave (Figure 2). The particle motion is periodic and characterised by its amplitude, its period (or frequency) and wave length. The amplitude is the difference between the maximum and the minimum position value (Figure 2). The period is the time necessary to achieve a whole cycle (Figure 2). The frequency is the inverse of the period (Figure 2). The wave length is the spatial period of the motion, that is to say, the minimum distance between two points in the same displacement from their equilibrium point (Figure 1).

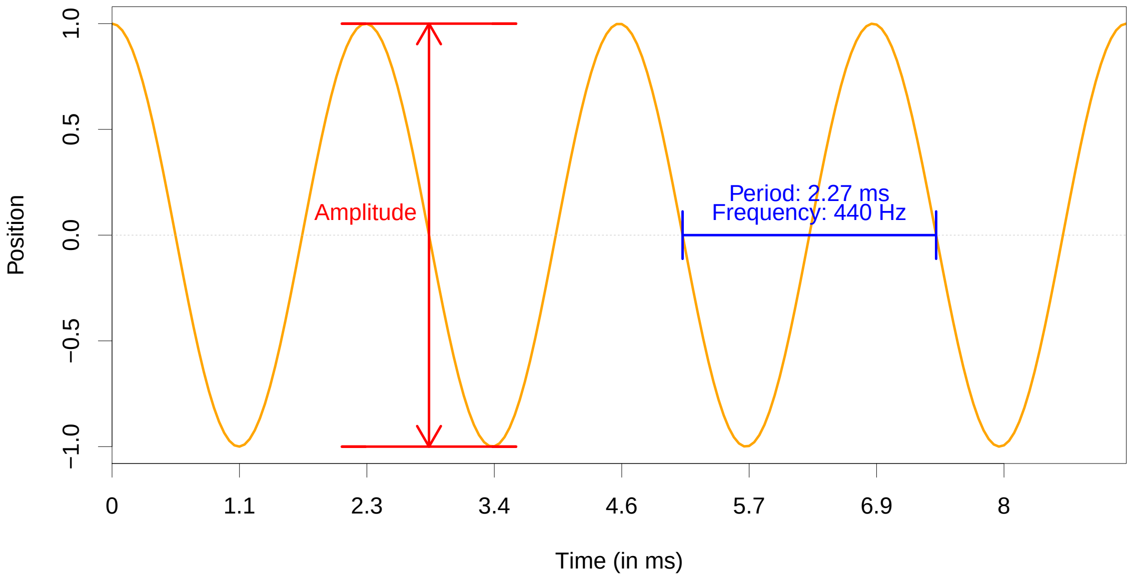

Figure 2 – **Motion of a particle in an acoustic field**. The particle oscillates around an equilibrium point at a frequency of 440 Hz, corresponding to the musical note A.

For a given sound, the perception of the amplitude correspond to the loudness of the sound which can be loud or quiet. Perception of the period or frequency corresponds to the pitch: high pitched sounds are high frequency sounds and low pitched sounds, low frequency sounds.

The amplitude of a sound is measured through the variation in pressure (p) induced by the sound to the fluid. Close to the vibrating source, in the volume called the near field, the wave propagation is not plane, meaning that particle velocity is not linearly linked to sound pressure (see below). To measure a sound source, it is usually recommended to avoid this volume with complex characteristics and to be placed in what is called the far field where the wave propagation is plane. The limit between the near and far field is estimated as the Fraunhofer distance:

$$d_f > 2\frac{D^2}{\lambda} \tag{1}$$

with d_f, the Fraunhofer distance, D, the diameter of the vibration source and λ the wavelength of the sound.

The intensity in acoustics is defined as the quantity of power delivered per surface unit. The intensity of a sound I equals to:

$$I = \frac{P}{S} \tag{2}$$

With P the power delivered by the sound to the medium and S the surface to which the power is delivered. The intensity of a sound can also be expressed in function of the sound pressure as $I = pv$ with p the pressure of the sound and v the particle velocity of the sound. The particle velocity of a sound in the far field is equal to

$$v = \frac{p}{z} \tag{3}$$

with p the sound pressure and z the impedance of the medium. The impedance of the medium depends on the density of the medium and the sound celerity in the medium: $z = \rho c$, with ρ the medium density and c the celerity of sound in the medium.

Therefore,

$$I = pv = \frac{p^2}{z} = \frac{p^2}{\rho c} \tag{4}$$

Two metrics are usually used to quantify sounds: the sound pressure measured in Pascals (Pa) and the sound loudness measured with a relative scale called decibels (dB). Decibels are a logarithmic scale based on human perception of sounds. Decibels are measured according to a reference intensity or pressure:

$$A = 10 \times log_{10}\Big(\frac{I}{I_0}\Big) \tag{5}$$

with A the amplitude of a sound in dB, I the intensity and I_0, the reference intensity. Typically, sound is measured as a pressure. According to equation (4),

$$A = 10 \times log_{10}\Big(\frac{p^2}{p_0^2}\Big) = 20 \times log_{10}\Big(\frac{p}{p_0}\Big) \tag{6}$$

An amplitude in dB is always relative to its reference. Typically, the reference p_0 in air is 20 μPa, which corresponds to the human threshold of hearing at 1 kHz, and 1 μPa in water. Table 1 presents a few examples of sounds and their amplitude measured in decibels.

Most of the time the perception referential is the human ear which on average can hear "sounds" ranging from 20 Hz to 20 kHz. Therefore, acoustic waves below this threshold are called infra-sounds while acoustic waves above are called ultrasounds.

Table 1 – **Examples of sound sources and their amplitudes at 1 m** (source: wikipedia).

Source	**Amplitude (in dB re 20 μPa)**
Normal conversation	40–60
TV (set at home level)	60
Vuvuzela horn	120

Methods for recording and analysing sounds

Acoustic sensors

To measure, record or amplify sounds, some form of acoustic sensor is necessary, typically converting pressure variation into an electrical signal. Such sensors can be broadly categorised according to the physical medium in which they operate – *microphones* in air and other gases, *hydrophones* in water and other fluids, and *geophones* in the substrate; and also according to their mechanism – 'contact' sensors that use piezoelectric effects, 'dynamic' sensors that use electrodynamic transduction, and 'condenser' sensors that use electrostatic effects. Most microphones are either dynamic or condenser types (with condensers being the most commonly used in bioacoustics). Hydrophones and geophones are mostly contact sensors.

Condenser microphones are composed of a mobile membrane (or diaphragm) and a fixed back plate. A polarizing voltage is applied to the microphone backplate via a high resistance. The diaphragm and back plate constitute a capacitor (historically known as a condenser). Sound waves makes the diaphragm vibrate creating variation in the distance between the diaphragm and the back plate. This variation of distance modifies the capacitance of the capacitor formed by the diaphragm and the back plate, which is converted into an electrical signal (Figure 3). The higher the amplitude of the sound, the larger the displacement of the diaphragm and therefore the higher the amplitude of variation of the electrical signal.

Dynamic and condenser microphones can be further classified as omnidirectional or directional, according to whether they detect variations in absolute pressure, or variations in pressure gradient along a particular axis. Omnidirectional microphones, which detect absolute pressure, have one side of the sensing membrane exposed to the air (or other medium) whilst the opposite side is enclosed in the body of the microphone isolated from external pressure variations - so that the membrane responds to the pressure difference between inside and outside of the microphone body regardless of the direction of incidence of the pressure waves. Directional microphones have both sides of the sensing membrane exposed to the acoustic medium, and so the membrane moves in response to the pressure gradient, with on-axis incident plane waves producing maximum signal amplitude, and orthogonally incident waves producing no signal.

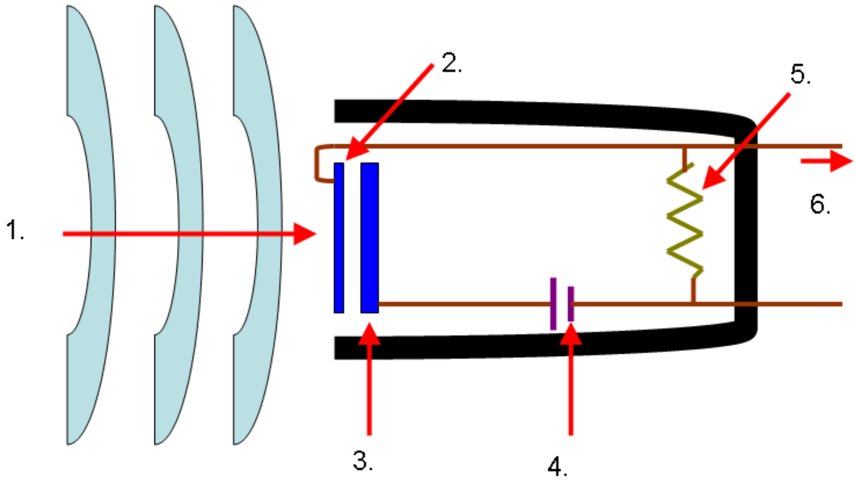

Figure 3 – **Diagram of a condenser microphone**. 1. Sound Waves, 2. Diaphragm, 3. Back Plate, 4. Battery, 5. Resistor, 6. Audio Signal (Source: wikimedia).

Hydrophones, in contrast, contain a piezoelectric element enclosed in a waterproof capsule (Figure 4). The piezoelectric element is made of a material which has the property of transducing mechanical deformations into electric impulses - typically special ceramics are used in hydrophones. Hydrophones are almost always omnidirectional (although directional sensing with piezoelectrics is possible), and the distinction between omnidirectional *vs* directional sensors is usually framed in terms of pressure *vs* particle velocity. Where directional and spatial information is desired, an array of hydrophone is typically deployed.

In the near field or in particular environments such as a finite volume of water enclosed by the walls of an aquarium, the relationship between particle velocity and pressure is non linear, and in these cases laser vibrometry can be used to measure particle velocity. Finally, to measure substrate-borne vibration, several alternatives are available, such as laser vibrometry, accelerometers or magnetic cartridge (electric transducer in vinyl players).

After the conversion of the acoustic vibration into an electrical signal, the electrical signal is usually translated back and recorded into an accessible format for storage and later use. There are two recording types: analogue and digital. The analogue system enables a continuous recording of the sound wave. Analogue recorders are old-fashioned recorders such as gramophone or tape recorders. Gramophones translate the electrical signal into a three dimensional pattern usually stored on a vinyl disc. Tape recorders translate the electrical signal into a magnetization pattern on a magnetic band. For most modern scientific purposes digital representations are used. The fundamental attributes

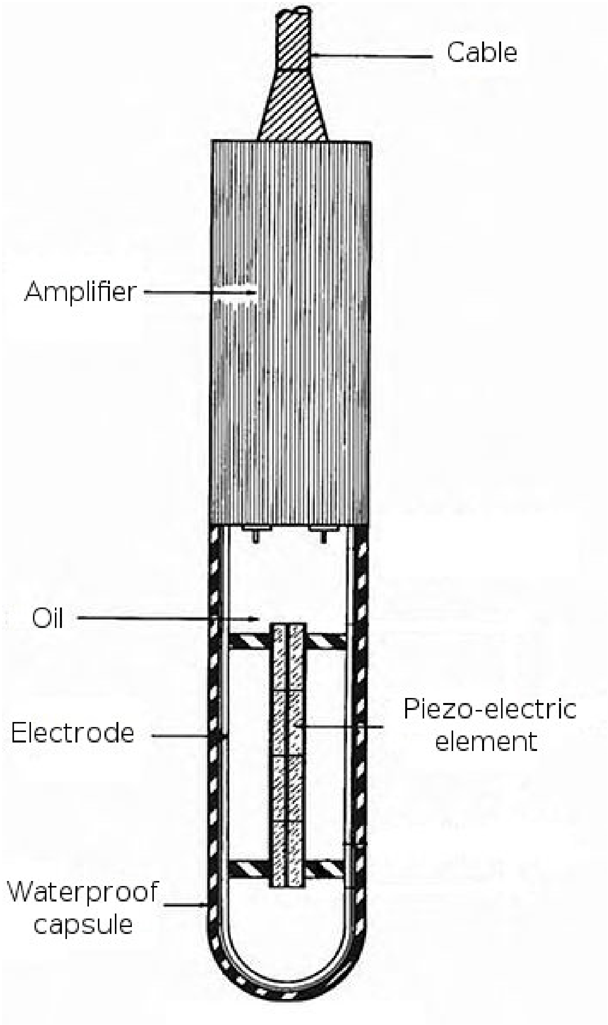

Figure 4 – **Diagram of a hydrophone** (Source: wikimedia).

of a digitisation scheme are its (i) sampling rate – how many discrete measurements (or samples) are taken of the continuous signal per second, and (ii) bit depth - the resolution in bits to which amplitude levels are quantised, for example 16 bits allows for $2^{16} = 65536$ distinct levels.

Digital signals require less storage space and are easier to manipulate and analyse with a computer software. However it is important that sampling rate and bit depth are set appropriately for the intended task. Recording at a sampling frequency of f Hz (meaning that f samples are recorded per second), allows the analysis of sounds of with a maximum frequency of f/2, called the Nyquist frequency (Figure 5). As shown in Figure 5, if the frequency of the analysed sound exceeds f/2, the periodic oscillation can be overlooked, or worse create frequency artefacts through a process known as aliasing. Therefore the sampling frequency must be at least twice as high as the highest frequency of the recorded sound, and it is even recommended to be at least 2.5 times the frequency of the sound to ensure the capture of the whole signal.

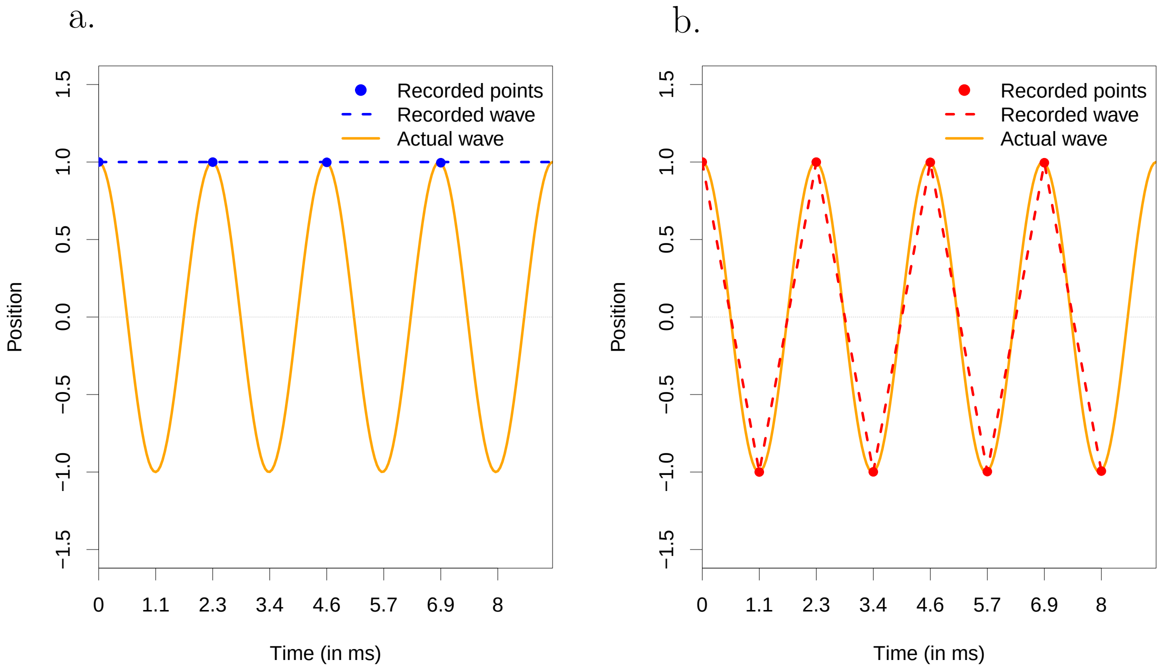

Figure 5 – **Example showing the under-sampling issue on a 440 Hz sound (the musical A)**. a. Signal recorded with a sampling frequency of 440 Hz, b. Signal recorded with a sampling frequency of 880 Hz.

Signal analysis

Signal analysis is a trans-disciplinary scientific domain, enabling to extract and investigate elements considered as signals. One of the main challenges is to extract a signal out of the background noise. To visualise and analyse sounds, three main representations of the sound are used: the oscillogram, the power spectrum and the spectrogram.

The oscillogram is a representation of the waveform (Figure 6a). It represents the amplitude in function of the time. It enables to visualise and measure the amplitude of sounds and their variations (amplitude modulation) and the temporal patterns (*e.g.* sound duration, inter-sound interval, repetition rate).

The power spectrum corresponds to the representation of the amplitude in function of the frequency (Figure 6b). This representation is obtained thanks to the Fourier transform. The Fourier transform, initiated by the mathematician Joseph Fourier during the 19th century, decomposes any time series into a linear combination of sines and cosines. The sine and cosine coefficients, or Fourier coefficients correspond to the amplitude of the each frequency. The spectrum enables to measure the frequency features of a sound such as dominant frequency and frequency band.

The spectrogram is a representation of the frequency spectrum in function of the time (Figure 6c). It is obtained with a short term Fourier transform (STFT) which

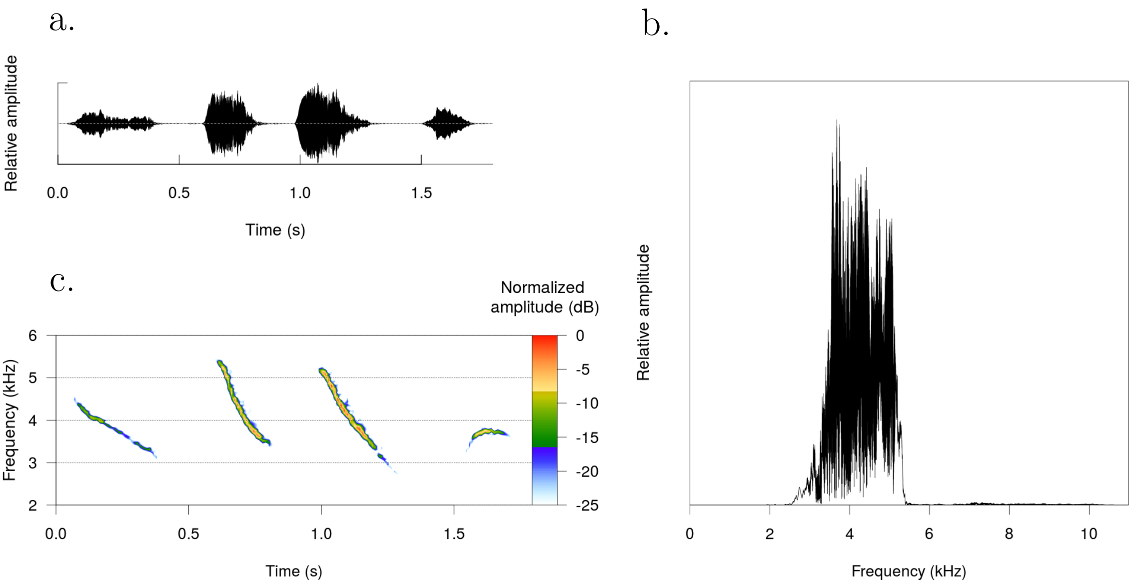

Figure 6 – **Different representations of a song of the rufous-collared sparrow (*Zonotrichia capensis*)**. a. Oscillogram, b. Power spectrum, c. Spectrogram, (Fourier window length: 512 samples, overlap: 50%, window type: Hanning). The song is composed of four notes lasting approximately 0.25 s each and separated by intervals of silence of 0.15 s. The frequency of the song is comprised between 3.5 and 5.5 kHz. The three first notes have a downward frequency modulation, from 5.5 to 3.5 kHz approximately, the last note is far less modulated, with a dominant frequency around 3.8 kHz.

computes the spectrum on successive time windows. The usual graphic representation of the spectrogram shows the frequency in function of the time. The amplitude of a frequency at a particular time is represented by the intensity or colour of the point in the image. Similarly to oscillograms, spectrograms enable to visualize the temporal pattern of the sounds, but the frequency information is added. It enables to visualize the frequency values and the frequency modulation in the signal along time.

Biological sounds

Living beings emit a tremendous diversity of sounds (Fletcher, 2007). A large variety of animals and even plants (Gagliano et al., 2012), occupying various environments emit sounds, among which birds, mammals, fishes, amphibians, crustaceans, insects, spiders and echinoderms (Figure 7).

Organisms produce sounds in various contexts. Sounds can be emitted intentionally, or as by-products of other behaviour. For example, the sound of a cow chewing is a by-product of the feeding behaviour, it is unintentionally produced, and the sound emitted does not have a specific function. On the other hand, a male frog call has a signal function. With this signal, the male aims at attracting females and repelling other males. Finally, some intentional sounds are auto-communication signal used by animals to orient

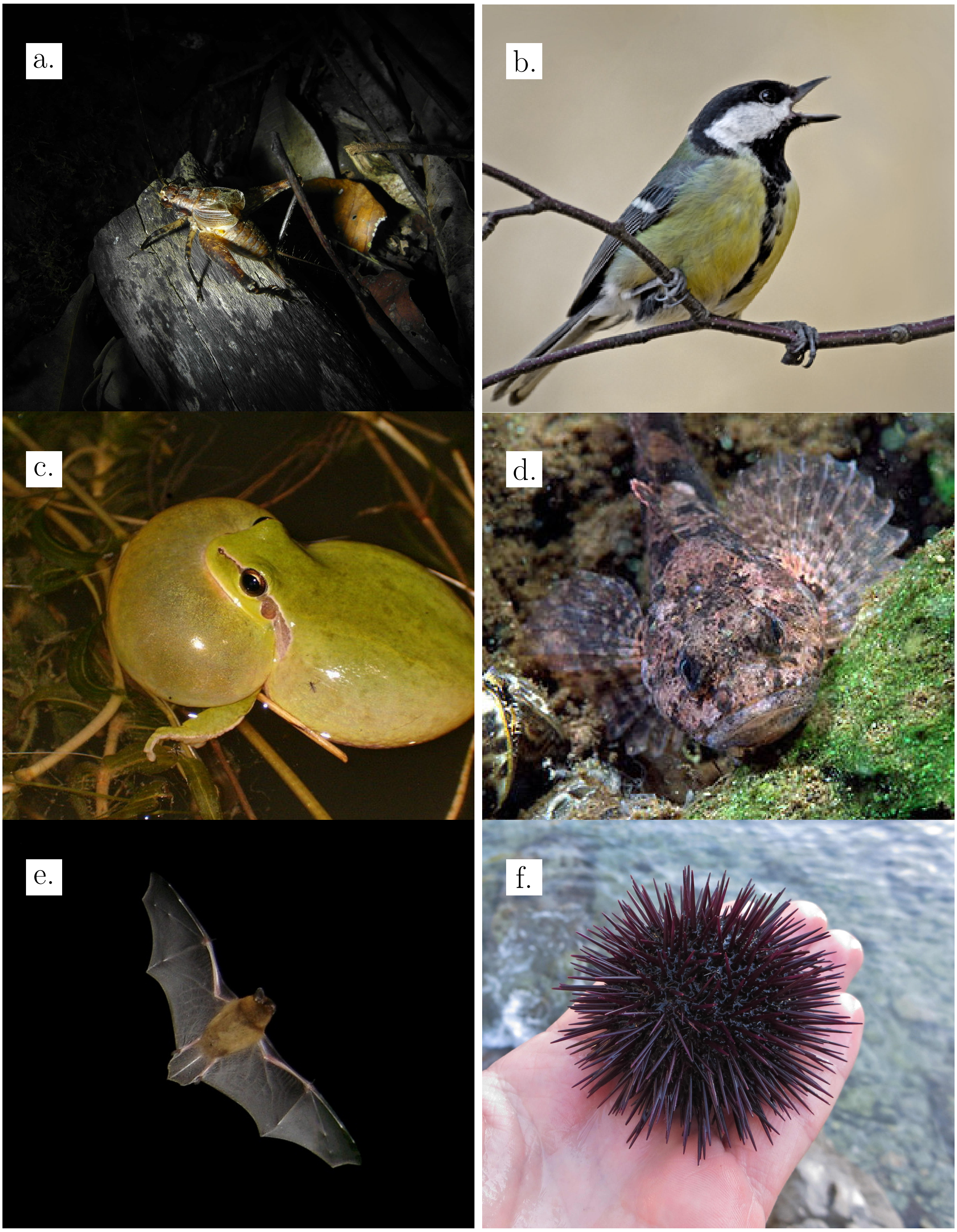

Figure 7 – **Diversity of sonic species**. a. Cricket, *Lebenthus sp.* (undescribed species, picture: Tony Robillard), b. Great tit, *Parus major* (source: wikimedia), c. Mediterranean tree frog, *Hyla meridionalis* (source: wikimedia), d. Common bullhead, *Cottus gobio* (source: wikimedia), e. Common pipistrelle, *Pipistrellus pipistrellus* (source: wikimedia), f. Purple sea urchin, *Paracentrotus lividus* (source: wikimedia).

themselves. This is the case of echolocation signals in toothed whales and bats mainly.

Similarly to musical instruments which produce sound by different mechanisms such as friction, percussion or constricted air flow, animals and plants emit sounds via different mechanisms and organs. Many animals such as insects or crustaceans produce sounds by friction of a rigid edge (the scraper) over a striated surface (the file). This mechanism is called stridulation. For example, crickets produce sound by rubbing a rigid edge of their right elytra on a striated area on the left one. Other animals, such as some birds, frogs and mammals emit sounds by vibration of a pair of membranes (either the lateral labia of the vocal organ, the syrinx, in birds or the vocal cords in the larynx in frogs and mammals) which are excited by a constricted flow of air. The vocal organ of birds is double, and a lot of species are able to alternate rapidly the use of the two sides to produce highly modulated sounds. Other species such as gorillas, river bullheads or some insects produce sound by percussion. Gorillas and river bullheads strike two body parts together, the fists on the chest and two cranial bones respectively. Some insects such as Plecoptera emit sounds by hitting leaves. Finally, many fish species produce sound by quick sonic muscle contraction. These sonic muscles are indeed the fastest muscles in the animal kingdom, they contract at rates reaching up to 280 Hz (Kever et al., 2014).

The diversity of organisms producing sounds with different mechanisms and in different contexts results in a huge diversity of sounds with various frequency and temporal features (Figure 8). Some animals produce infra-sounds such as elephants (*Loxodonta africana*: Langbauer Jr. et al. 1989, *Elephas maximus*: Payne et al. 1986) or Humpback whales (Clark, 2004, *Megaptera novaeangliae*). Other animals can produce ultrasounds such as bats (Fletcher, 2007), marine mammals (Fletcher, 2007) or crickets and ants (Robillard and Desutter-Grandcolas, 2004; Pavan et al., 1997). The loudest animals recorded so far are whales with amplitudes reaching up to 236 dB re 1 μPa at one meter for the sperm whale (*Physeter macrocephalus*, Møhl et al., 2003). Very quiet sounds emitted as signals include for example the wing vibrations emitted by the fruit fly, *Drosophila melanogaster*, during courtship displays which reach 36 dB re 20 μPa at 5 mm which is not perceptible by the human ear (Aubin et al., 2000).

Animal sound in the environment

Living organisms emit sounds in their environment. The environment is here considered as the sum of elements (biotic or abiotic) in which an organism survives and reproduces. The propagation of a sound in the environment is a complex phenomenon which is influenced by several factors.

In a three dimensional and homogeneous medium, a sound emitted at one point in space will propagate equally in all directions around this point, thus sound wave propaga-

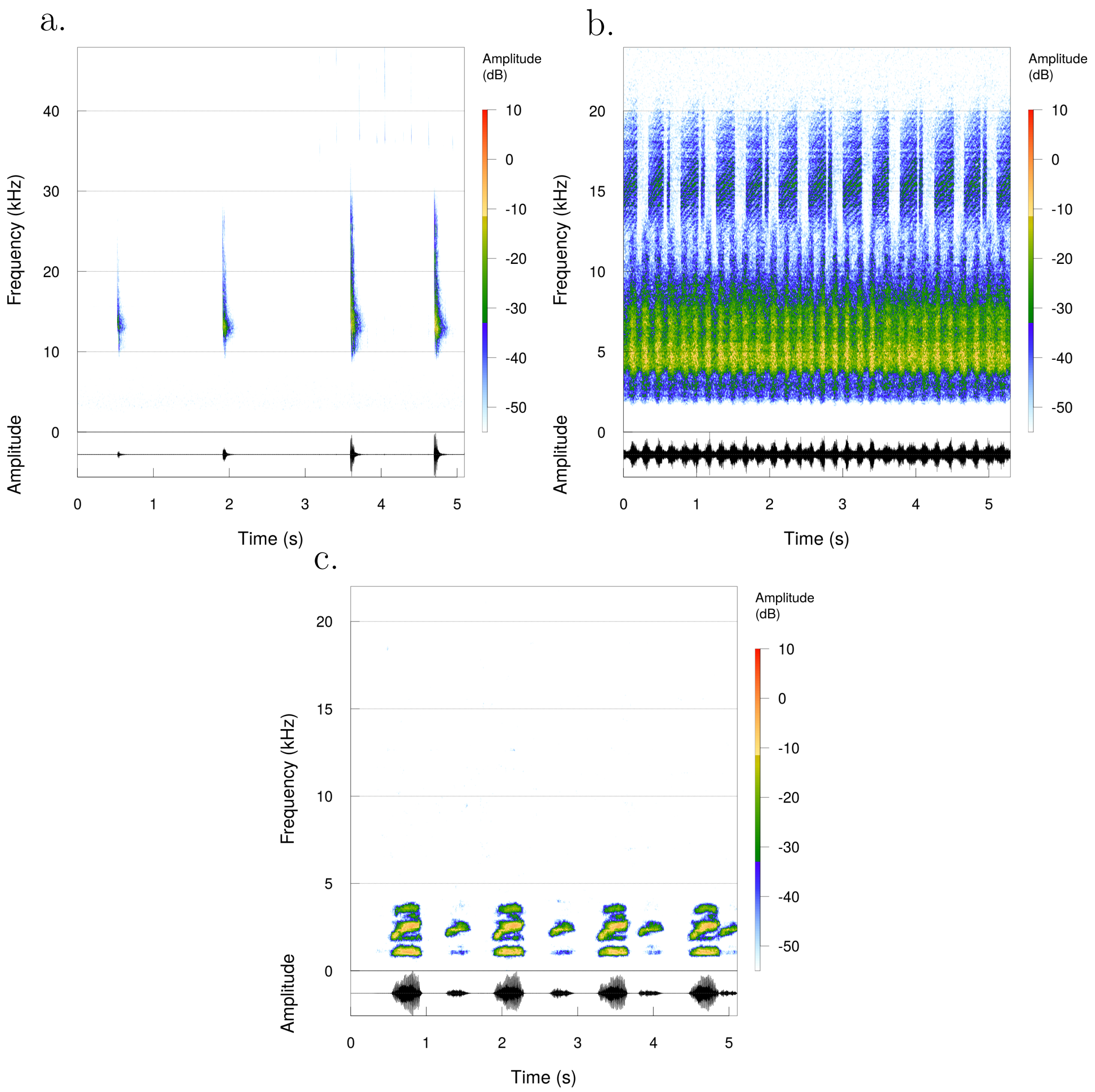

Figure 8 – **Diversity of animal sounds**. a. Social call of Kuhl's pipistrelle, *Pipistrellus kuhlii* recorded with Aurélie Laurent in Bayeux in September 2014, b. Chorus mixing two species of cicadas, *Tettigettula pygmea* and *Cicada orni*, recorded with Jérôme Sueur and Pierre Alexis Rault in Vidauban in June 2015, c. Two individuals of Mediterranean tree frog, *Hyla meridionalis* recorded in Bormes-les-Mimosas in June 2013.

tion is spherical. Assuming that the medium is ideal, *i.e.* it does not absorb any sound, then the total power is conserved. Each wave front describes a sphere containing the same uniformly distributed power: $P = P_0$, with P the power at any wave front and P_0 the power at the source.

Intensity I equals a power over a surface unit. Therefore:

$$P_0 = 4 \times \pi \times r_0^2 \times I_0 = 4 \times \pi \times r^2.I \tag{7}$$

with r_0 and r the radius of two spheres which are crossed by the sound ($4 \times \pi \times r_0^2$ and $4 \times \pi \times r^2$, the surfaces of the spheres), I_0 and I, the intensity at an area on the spheres of radius r_0 and r respectively and P_0 the power at the source. From equation (7) follows that as the surface of the sphere gets bigger (when the sound propagates), the intensity necessarily decreases. The expression of the intensity at one point in space at a distance r from the source can be expressed as:

$$I = I_0 \times \frac{r_0^2}{r^2} \tag{8}$$

Therefore, the attenuation of a sound when doubling the distance ($r = 2r_0$) from the source is doubled and equals to:

$$A = 10 \times log_{10}\left(\frac{I}{I_0}\right) = 10 \times log_{10}\left(\frac{r_0}{r}\right)^2 = 20 \times log_{10}\left(\frac{r_0}{2r_0}\right) = -6\ dB \tag{9}$$

This phenomenon is called spreading loss and explains why even without any absorption, the amplitude of the sound decreases with the distance from the source. According to equation (8), spreading loss is independent from the frequency of the sounds.

Spreading loss is not the only source of attenuation. The attenuation due to other sources than spreading loss is called excess attenuation. The first source of excess attenuation is absorption which applies to any homogeneous or non-homogeneous medium. Absorption results in a part of the energy of the sound dissipated as heating or rotational and vibrational relaxation of molecules (Wiley and Richards, 1982). This attenuation depends on the acoustic impedance of the medium, which itself depends on several environmental factors. The impedance of the air mainly depends on temperature and relative humidity. The impedance of the water mainly depends on temperature and salinity. In both media, the attenuation due to absorption is frequency dependent; high frequencies are more attenuated than low frequencies. This is why in a concert, the sounds that can be heard the furthest are the basses.

Natural environments, either terrestrial or marine, are far from homogeneous. They contain various medium boundaries such as air/water, air/vegetation, air/ground, water/vegetation, or water/ground. Such boundaries result in reflection, refraction and diffraction of sound waves. The size of the boundary as well as the impedance differential at the boundary have an impact on the transmission of the wave.

For example, due to the high impedance differential between air and water, most of the acoustic energy is reflected by the water/air surface. Only a very small proportion of the energy is refracted and therefore propagates in the water. This is why, underwater sounds are not easily heard in air and vice-versa. Diffraction happens only when the size of the object or slit is of the same order of magnitude as the sound wavelength. Refractions and reflections are negligible on objects smaller than the wave length of the sound. Therefore the propagation of the sound wave is only significantly impacted by obstacle of the same order of magnitude (diffraction) or bigger (refraction and reflection) than the sound wavelength. This explains why low frequency, which have large wavelength, are less diffracted and reflected by vegetation than high frequency.

Due to all these physical phenomena, some frequencies propagate better than others. This frequency dependent propagation of waves can result in distortions of sounds which are not pure tones (containing a frequency modulation or harmonics for example).

Depending on the propagation medium of the sounds, different effects are to be expected. Closed environments such as tropical forests result in important echoes and diffraction of sounds due to the abundance of foliage (Figure 9a). Therefore a sound does not travel as far in forests as in an open environment such as open ocean (Figure 9b), or a plain (Figure 9d). However plains and open oceans ma be more subjected to background noises such as wind or waves. Finally, a lake environment above water surrounded by mountains may result in echoes due mainly to the reflective nature of the water (Figure 9c).

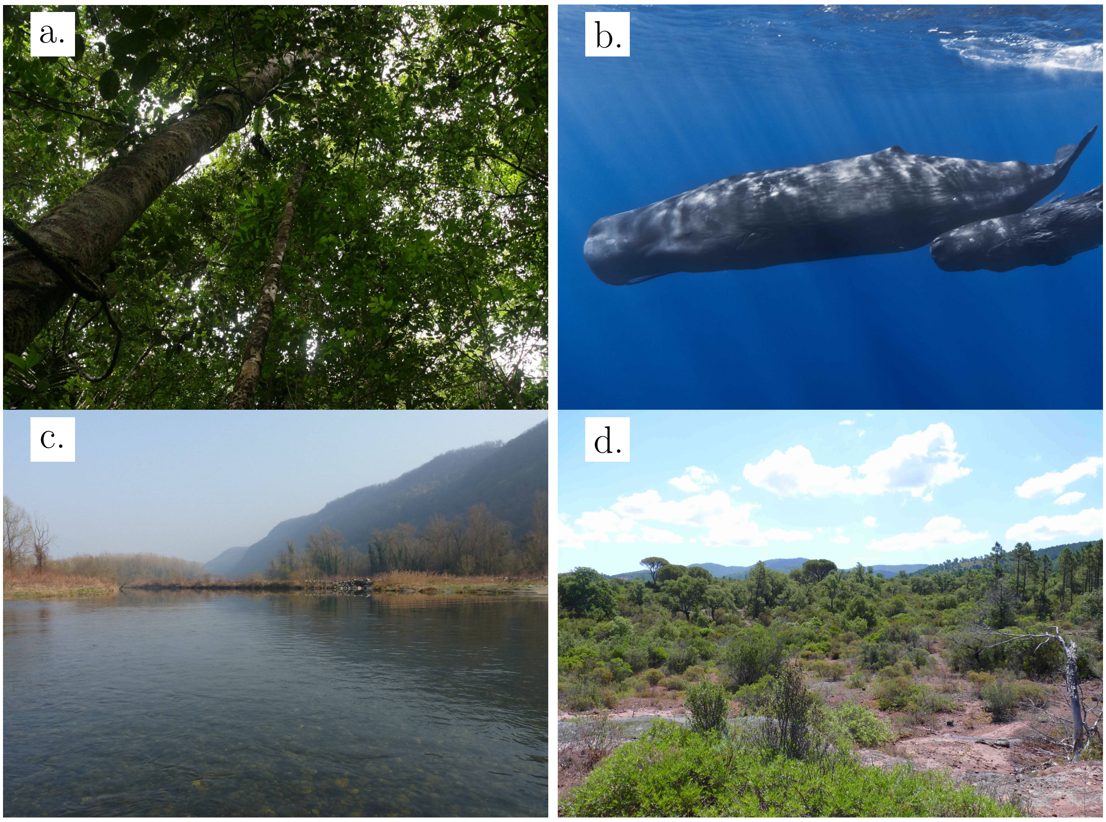

Figure 9 – **Diversity of natural environments**. a. Tropical forest (credit: Juan Sebastian Ulloa), b. Open water in the sea (source: wikimedia), c. Belley, Ain (01), France, River and mountainous environment. d. Plaine des Maures, Var (83), Mediterranean plain.

Studying the existing relationships between animal sounds and the environment is one of the main aims of an emerging scientific discipline studying biological sound, called ecoacoustics.

Ecoacoustics: Linking acoustics and ecology

Definition

Ecoacoustics is a recently emerged discipline, defined in Sueur and Farina (2015) as "a theoretical and applied discipline that studies sound along a broad range of spatial and temporal scales in order to tackle biodiversity and other ecological questions". Here, ecology is defined as organism-environment interactions, therefore including abiotic and biotic effects.

Sueur and Farina (2015) also define new study scales, homologous to classical ecological scales, applying to ecoacoustics: "The use of sound as a material from which to infer ecological information enables ecoacoustics to investigate the ecology of populations, communities and landscapes". An acoustic population, similarly to the ecological definition of a population, is a mono-specific assemblage of sounds at one location and time (Sueur and Farina, 2015).

Acoustic populations have mainly been studied in the scope of population monitoring in a wide range of animal groups such as marine mammals (Risch et al., 2014), birds (Laiolo et al., 2008), amphibians (Llusia et al., 2013a; Dutilleux and Curé, 2016) or insects (Jeliazkov et al., 2016).

An acoustic community is a collection of sounds produced by a set of organisms coexisting in a given habitat over a specified time (Gasc et al., 2013a). Acoustic communities were studied mainly in tropical forests (Rodriguez et al., 2014; Sueur et al., 2008). Finally, a soundscape, is defined as "the collection of biological, geophysical and anthropogenic sounds that emanate from a landscape and which vary over space and time reflecting important ecosystem processes and human activities" (Pijanowski et al., 2011). Most of the investigated soundscapes so far are terrestrial (*e.g.* Joo et al., 2011; Mullet et al., 2015; Gage and Axel, 2014) although a few aquatic soundscapes have also been explored (*e.g.* Tonolla et al., 2010; Rossi et al., 2016; Ruppé et al., 2015).

Bioacoustics is the discipline studying acoustic communication systems based on the information theory (Shannon, 1948). The information theory conceptualises signal exchange process as an emitter producing a signal which is transmitted through a propagation channel and then decoded by the receiver (Figure 10). Although bioacoustics and ecoacoustics are not fully disjoint disciplines, ecoacoustics adopts a new perspective by looking into the ecological signification of sounds produced in the environment. Ecoacoustics investigates the link existing between environmental sounds and the environment in a theoretical perspective, to understand the structure, dynamics and diversity of acoustic populations, communities or landscapes and in an applied perspective to develop and standardise new methods to monitor environments with acoustics.

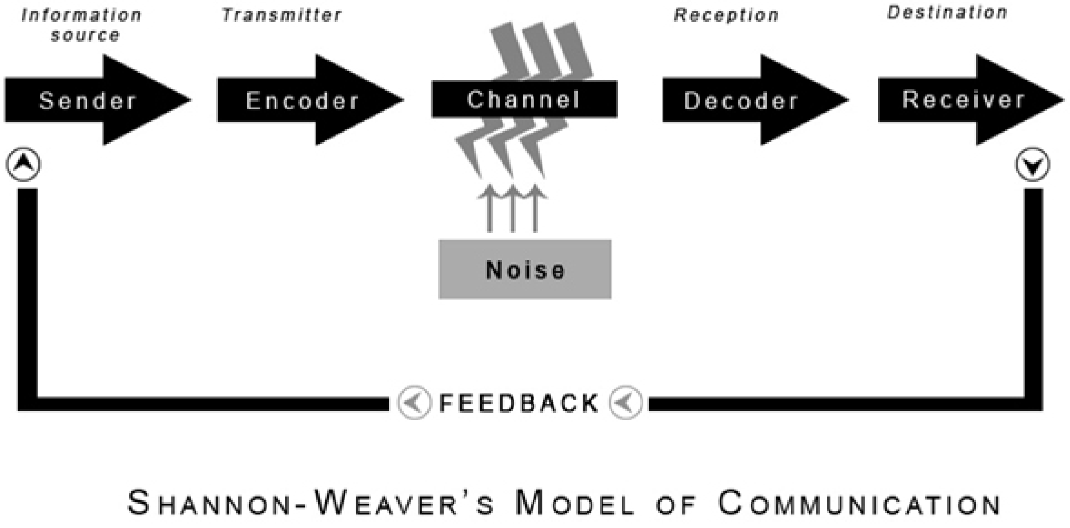

Figure 10 – **Shanon-Weaver model of information channel** (after Shannon, 1948)

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Sampling methods in ecoacoustics: Passive Acoustic Monitoring

Passive Acoustic Monitoring (PAM) is a sampling method which consists in recording or listening to sounds in the environment to survey acoustic population, acoustic communities or soundscapes. PAM can be applied to various systems to tackle various ecological questions including surveying species distribution, abundance and diversity, investigating temporal structures and phenology, and assessing responses to environmental disturbances (Blumstein et al., 2011). PAM can be achieved by several methods ranging from direct aural identification (Garcia-Rutledge and Narins, 2001; Hutto and Stutzman, 2009), focal recordings (Krause, 1993) to long term autonomous recordings (Zimmer, 2011; Duarte et al., 2015; Gasc et al., 2013b). We focus here on the latest: PAM with autonomous recorders connected to a set of microphones or hydrophones in an environment (Blumstein et al., 2011). This section highlights the advantages and the methodological challenges faced by this approach for the collection, management and analysis of acoustic samples.

Sample collection for PAM

The two main challenges for acoustic sample collection in ecoacoustics are: i) robustness and ii) autonomy.

Natural environments are subject to several disturbances such as rain, wind, wild animals, humidity or extreme temperatures which can impair the quality of the recordings (Depraetere et al., 2012). Electronic devices used as acoustic sensors and recorders are usually delicate, they function properly under restrained temperature and relative humidity conditions. Therefore, to record in environments such as tropical forests or mountains where weather conditions are extreme (*e.g.* heavy rains or snow, extreme temperature), ecoacoustic recorders are required to be robust and weather proof (Blumstein et al., 2011). Several weather proof devices have been developed (Figure 11), they include a water-proof enclosure for the recorder and robust microphones or hydrophones.

Figure 11 – **SM2 autonomous recorders installed in the field**. The green boxes contain the autonomous recorders and are connected with a water-proof plug to a hydrophone. a. Four SM2 recorders (cases opened) used to record a Mediterranean pond. b. One SM2 installed in a secondary channel of the river Rhône in Brégnier-Cordon (picture: Diego Llusia).

PAM can be used in remote locations and for extended periods of time. Therefore, another challenge for the recording devices is autonomy in energy, storage, and recording.

- Storage has improved drastically in the past 20 years. Nevertheless the recording of high frequency sounds such as those produced by bats or toothed whales require very high sampling rates inducing tremendous storage consumption. One solution to this storage issue can be to stream the recorded data into another location with higher storage capacities. For example, in the scope of the ARBIMON project, monitoring tropical forests in Puerto Rico and Costa Rica, the data are streamed to a server via an antenna (Aide et al., 2013).

- Energy consumption can also be limiting. Low consumption recorders have been developed to palliate this limitation. Another solution can be to use a locally available power source such as solar or hydropower (Aide et al., 2013).

- Recording autonomy is advantageous to avoid human intrusion and reduce work force requirements. Indeed several autonomous recorders have been developed which enable to set different recording schedule (Farina et al., 2014). Indeed, according to the targets, the recording schedule and thus sampling collection may differ largely, going from whole day for soundscape analysis, to dusk and dawn for birds or night only for bats.

PAM is an advantageous collection method compared to classical sampling methods as it requires less work force than manual and physical sampling, enables to survey at larger spatial and temporal scales, and is non-invasive.

The non-invasive character of PAM has two main advantages:

- It avoids the flight bias of classical surveys. Indeed several sampling methods imply to capture the individuals with nets or traps, resulting in the easiest individuals being captured which may not represent accurately the overall population, community or ecosystem

- The surveyed habitats and biological communities are not impacted by the sampling which can result in direct death (for invertebrates and plants mainly) or by physical injuries or stress.

The main collection challenges of PAM have been overcome. However, the sample management and analysis are currently still under investigation.

Sample management for PAM

PAM generates huge datasets. As shown previously, thanks to recent improvements, data storage is not really a limiting issue any more. However, managing and archiving such datasets requires some organisation and planning. Acoustic data are always associated to essential meta data such as time, date, location, or recorder brand. The loss of those meta data results in the loss of scientific value of the recordings. All these data can not be all stored in the sound file, it is therefore necessary to conserve the link between recorded samples and their respective meta data. Different architectures have been proposed to ensure the archiving of PAM samples with their meta data (Roch et al., 2013). Moreover, several sound libraries such as the sonothèque (https://sonotheque.mnhn.fr/) in the MNHN or the Macaulay library (http://macaulaylibrary.org/) of Cornell University, ensure the conservation of recordings via storage on computer servers.

Traditional sampling methods are faced with similar issues to conserve the meta data associated with the samples. Acoustic data has the advantage of being mostly digital files which can be copied, shared and analysed without destruction while in the case of macro-invertebrate samples, for instance, individuals can get degraded over time, and may have to be damaged for identification (*e.g.* genitalia extraction).

Sample analysis for PAM

The analysis of large datasets generated by continuous recordings is a real challenge for three main reasons: i) the size of the datasets, ii) the uncontrolled level of ambient noise and iii) the biases of acoustic samples

To analyse PAM samples, two main approaches can be undertaken: manual or automatic. The manual approach consists in a human operator analysing the recorded files. This time-consuming process is usually a pre-requisite to further analysis. However when the quantity of data is very high, down sampling or using automatic approaches is mandatory. The automatic approach consists in using computer tools to analyse automatically the acoustic data. There are several types of automatic tools depending on the scale of the study. At the level of acoustic population, automatic detection of the sounds using spectral and temporal features can be used to asses the presence-absence and potentially abundance of a given species (Ulloa et al., 2016). At the level of the acoustic community, several acoustic diversity index have been developed to assess acoustic diversity within the recordings (Sueur et al., 2014). Finally at the level of the soundscape, several index based on frequency band analysis have been developed to quantify the relative importance of biophony, geophony and anthropophony (Sueur et al., 2014).

Automatic detection in natural environments is faced with unpredictable and unknown sources of noise. Indeed, detecting the animal signals lost in the surrounding environmental sounds constitutes an important challenge for signal analysis. Different methods to reduce the effect of noise are used. In many studies, rainy and windy recordings are discarded (Gasc et al., 2013b) or controlled for (Depraetere et al., 2012). Other studies apply frequency filters (Depraetere et al., 2012) or noise reduction procedures (Potamitis et al., 2014) to the files in order to improve the quality of the recordings.

Automatic detection of acoustic events is highly advantageous compared to other classical methods as it requires a low work force and no particular skills if the detection method is operational. In contrast, taxonomic identification of macro-invertebrates requires a relatively high level of expertise and is time consuming.

All sampling methods are biased in a way or another. PAM is limited to animals emitting sounds. This has to be taken into account for instance when studying a population in which only males emit sounds or in a community including non soniferous species. This step of standardisation of acoustic recording has not been fully explored yet even though deriving links between ecology and acoustics also implies testing the link between species diversity, abundance and composition and sounds.

Drawing links between ecology and acoustics

Several studies have looked at different aspects of the link between ecology and acoustics. The first studies have mainly focused on the soundscape scale, deriving links with land use (Duarte et al., 2015; Joo et al., 2011; Kuehne et al., 2013) or ecosystem condition (Fuller et al., 2015; Tucker et al., 2014) for instance. Other studies have looked at patterns of acoustic diversity (Pieretti et al., 2011; Rodriguez et al., 2014; Towsey et al., 2014), or at links between acoustics and ecology at the level of the community (Malavasi and Farina, 2013; Gasc et al., 2013a) or population (Llusia et al., 2013a).

Although many links between ecology and acoustics have been drawn at different ecological scales, the processes behind these patterns are still rather mysterious.

In ecology, the interaction of deterministic and random phenomena shape natural population, communities, and ecosystems (Begon et al., 2006). Deterministic processes include inter/intraspecific interactions such as competition for a shared resource. In ecoacoustics, the resource shared between individuals and species is the acoustic space. The acoustic space is defined in three dimensions: space, time and frequency. Considering that this resource can be limiting and limited, the acoustic space may constitute a selection pressure and impact the evolution of sound production by organisms using and sharing this resource.

As we explored previously in the section *Animal sound in the environment*, different environments offer different sound transmission properties. The acoustic space of each environment is therefore different and the acoustic characteristics of the environment are expected to apply acoustic filters on the intentional sounds produced by animals (Morton, 1975). As a consequence, the organisms occupying a given environment are thought to have evolved to fit into the acoustic space of this environment. This means that sound features have evolved an optimised transmission space according to their function. This hypothesis is called the Acoustic Adaptation Hypothesis (AAH, Morton, 1975). Under this hypothesis, acoustic populations occupying environments with different acoustic spaces (*e.g.* close *vs* open environments, noisy *vs* quite environments) are expected to diverge. However acoustic populations in the same acoustic community, and therefore the same environment, are expected to converge due to similar transmission constraints.

Each species has specific sound productions which can be located at given positions in space, time and frequency. Each species therefore occupies a specific position within the acoustic space which correspond to its acoustic niche. As the resource is shared between different individuals, species and environmental sounds, sounds may overlap potentially resulting in masking. Inter and intra-specific competition for the acoustic space might therefore occur (Krause, 1987). Similarly to the concept of the Hutchinsonian niche (Hutchinson, 1957), this competition is thought to result in the partitioning of the resource, here the acoustic space (Krause, 1987). This hypothesis is called the acoustic niche hypothesis (ANH, Krause, 1993). Under this hypothesis, acoustic populations in the same acoustic community (same environment) are expected to diverge to avoid the cost of masking.

These two hypotheses apply opposite pressures on the evolution of sounds of a given acoustic community: under the ANH sounds should be over-dispersed, reducing the potential costs of masking while under the AAH, sounds should converge towards an optimum of transmission. The investigations of acoustic populations and communities have revealed evidences for both of these hypothesis.

For example, at the acoustic population level, support for the AAH has been found by studying two subspecies of song sparrows (*Melospiza melodia heermanni* and *Melospiza melodia fallax*) that occupy a continuum of habitats ranging from closed to open. Although the two subspecies had overlapping ranges, *M. m. fallax* was found preferentially in open habitats while *M. m. heermani* occupied vegetated habitats. Several differences were noted in the songs of the two subspecies: *M. m. heermanni*, the subspecies of closed habitat produced lower pitch and longer notes. A linear relationship between habitat characteristics and song features was found (Patten et al., 2004, Figure 12). Thus, lower frequencies, less affected by obstacles such as vegetation, and longer notes, less affected by the echoes and scattering, are more adapted to the transmission in closed environments. Although there are several examples similar to this one (Jiang et al., 2010; Slabbekoorn and Peet, 2003), the diversity of sounds found in several acoustic communities suggests that it is not the only process at hand.

At the acoustic community level, both the AAH and ANH have received support. Tobias et al. (2014) showed that acoustic communities of tropical bird were less divergent than expected by chance which could stem from the constrains of tropical forest environments. In contrast, several studies have found that acoustic communities found in an environment are partitioned in space (Villanueva-Rivera, 2014, Figure 13), time (Stanley et al., 2016) or frequency (Villanueva-Rivera, 2014, Figure 13). Finally, no specific pattern could be identified in some acoustic communities such as in cricket communities (Schmidt et al., 2016). These contrasted results suggest crossed-effects of the processes predicted by both hypothesis.

The processes behind the link between acoustics and ecology are still under debate

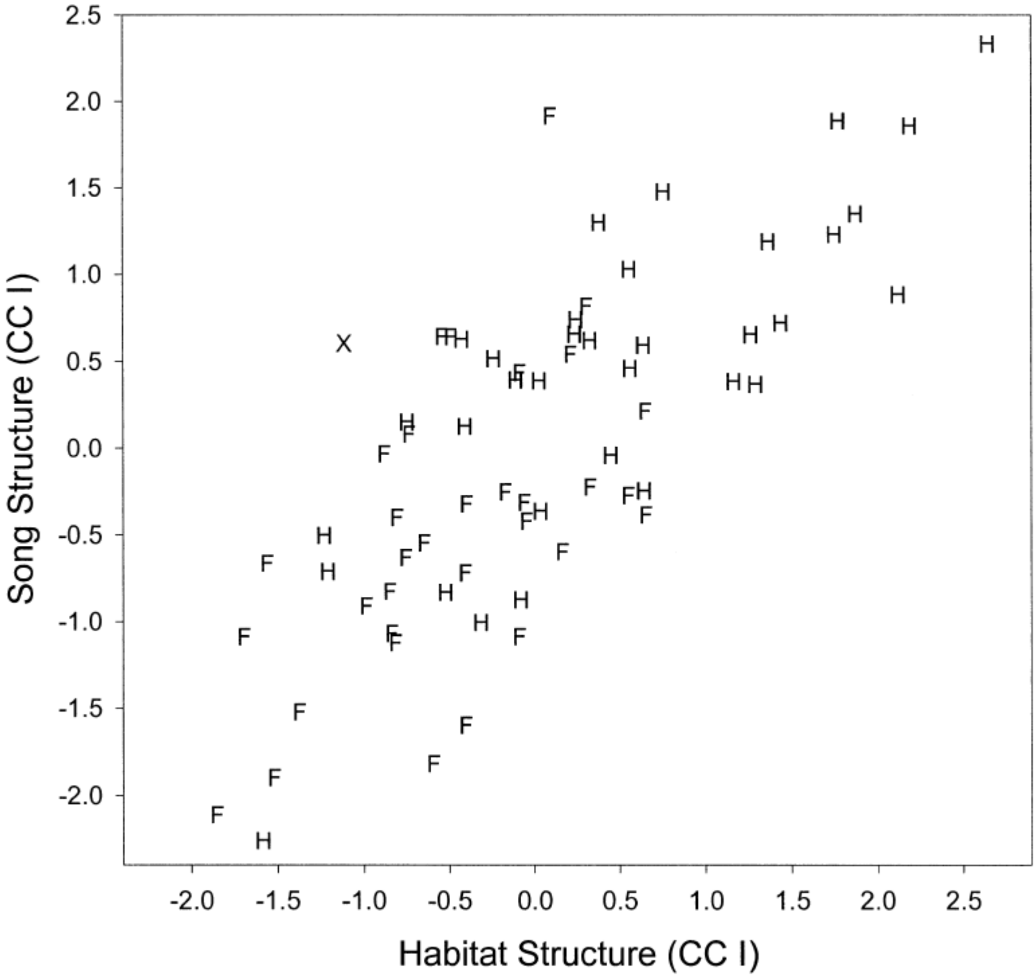

Figure 12 – **Relationship between habitat structure and song structure**. Habitat structure is the first axis of a multivariate analysis based on structure a composition of the vegetation. Similarly, song structure is the first axis of a multivariate analysis based on frequency, duration and cadence of the song and the notes. H : *Melospiza melodia heermanni*, F: *M. m. fallax*, X: hybrid. Higher vegetation density is associated with lower-pitched and more widely spaced song elements (Patten et al., 2004).

and investigation in various ecosystems. It appears that studying ecoacoustics at the community or population scales should increase our understanding of these processes.

Ecoacoustic studies have so far investigated several environments, ranging from terrestrial habitats such as tropical and temperate forests (Depraetere et al., 2012; Rodriguez et al., 2014; Malavasi and Farina, 2013), urban areas (Pieretti and Farina, 2013), plains (Mullet et al., 2015) to marine habitats such as open ocean (Parks et al., 2014; Ruppé et al., 2015), coral reefs (Bertucci et al., 2015), coastal waters (Felisberto et al., 2015; McWilliam and Hawkins, 2013), however one main environment seems neglected: freshwater environments. These environments with complex ecological functioning may be interesting systems for the investigation of the link between ecology and acoustics.

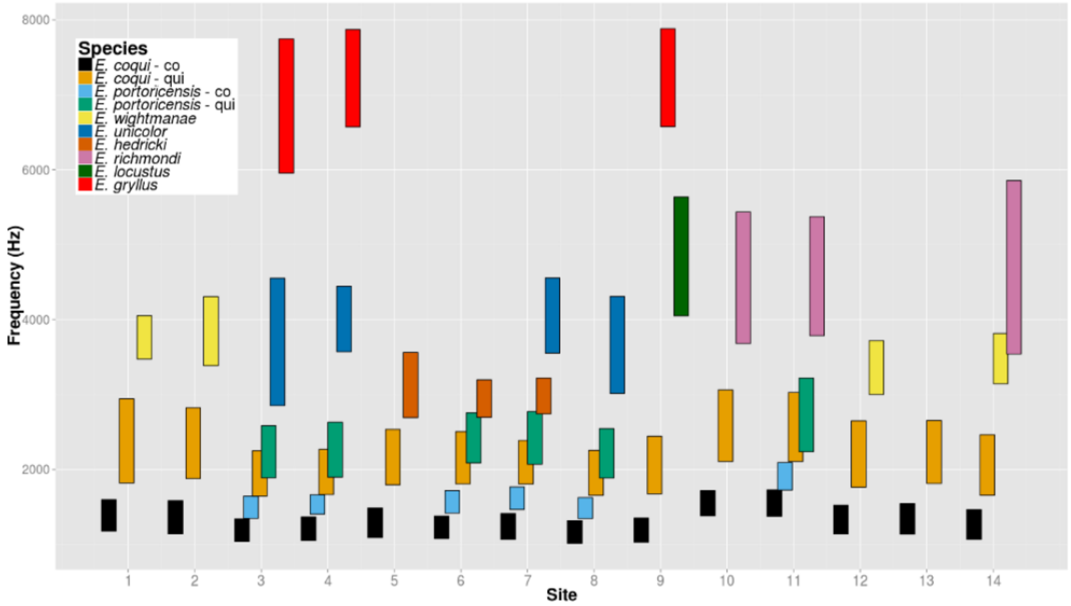

Figure 13 – **Frequency bandwidth of seven "Coqui" frog species** (genus *Eleutherodactylus*) at 14 sites in Puerto Rico. Both *E. coqui* and *E. portoricensis* have two notes, known as "co" and "qui", that were measured separately (Villanueva-Rivera, 2014).

Freshwater habitats

Importance and diversity of freshwater environments

Although fresh waters represent only 0.01 % of the world's water and about 0.8% of the earth surface, they host about 6% of the total animal and plant species described (Dudgeon et al., 2006; Abell et al., 2007). Besides constituting a tremendous source of biodiversity, freshwater environments are also necessary to the survival of many terrestrial species as they provide drinking water. Finally, freshwater environments are highly important to humans for ecosystem services (waste disposal), biodiversity and economic services (*e.g.* fisheries, hydroelectricity).

Freshwater environments are highly diverse in size, nature and functioning and correspond to three main categories of ecosystems (Silk and Ciruna, 2005, Figure 14):

- Standing-water ecosystems (*e.g.* lakes and ponds);
- Flowing-water ecosystems (*e.g.* rivers and streams);
- Freshwater-dependent ecosystems at the interface with terrestrial ecosystems (*e.g.* wetlands and riparian areas).

Freshwater environments are highly threatened, mainly because of the intensification and expansion of human activities (Dudgeon et al., 2006). In Latin America and the

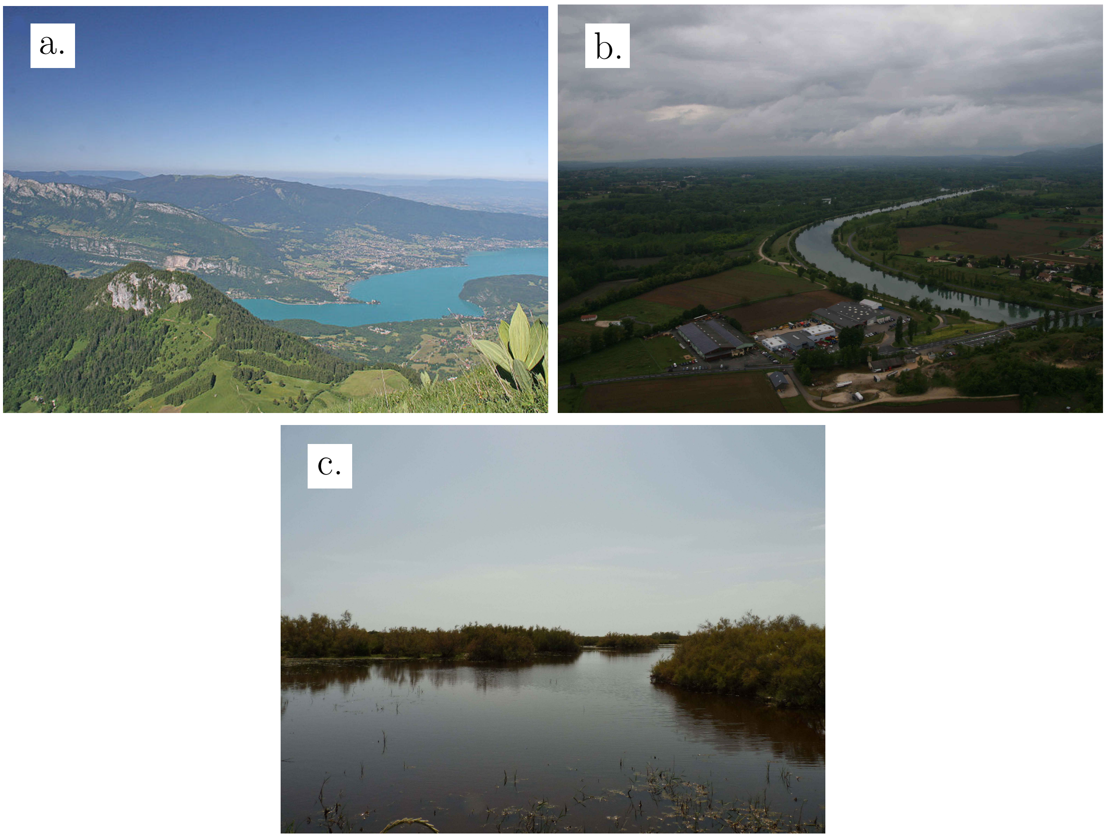

Figure 14 – **Examples of freshwater habitats**. a. Lake of Annecy (source: wikimedia), b. Rhône River, c. Pond in a wetland in Camargue

Caribean, 80 % of the freshwater ecosystems are considered critically endangered or vulnerable (Olson et al., 1998) and in Europe, Hull (1997) estimated that depending on the area, 40 to 90% of the ponds disappeared during the 20th century. Moreover, the rates of species extinction in freshwater environments are estimated to be on average five times higher than in any other groups of species (Silk and Ciruna, 2005). The main factors threatening these environments can be divided in five categories:

Over-exploitation Mainly affects fishes, reptiles or amphibians.

Water pollution By run-off waters are industrial waste.

Flow modification Mainly induced by dams.

Invasive species

Destruction or degradation of habitats Direct effects such as excavation for sediment extraction or indirect effects such change in the surrounding habitats can alter the sediment deposition regime.

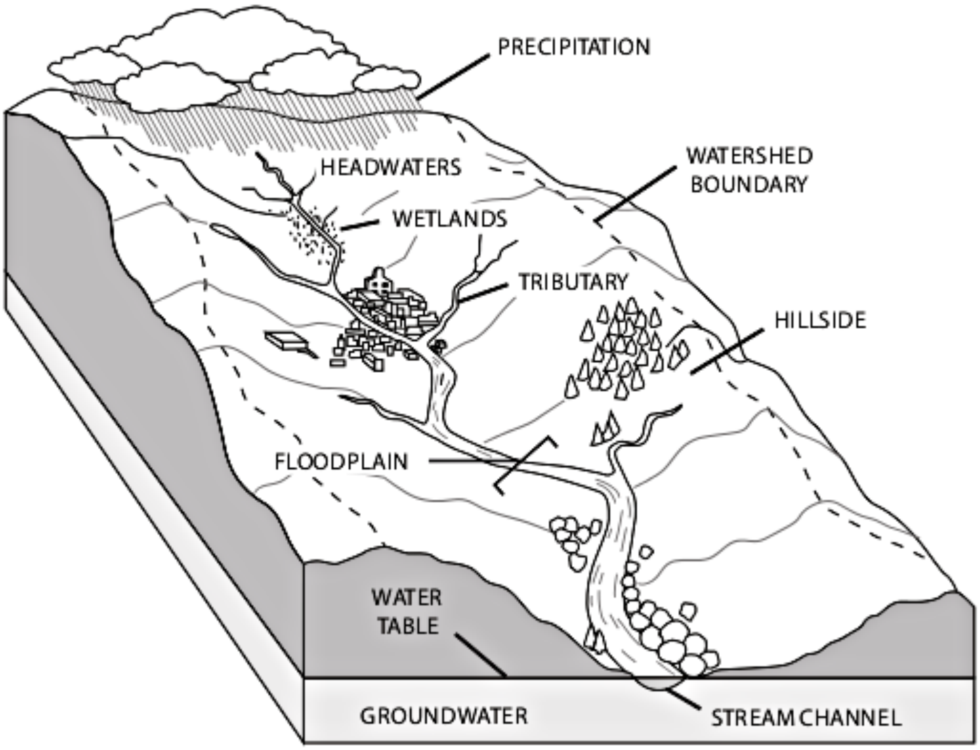

Figure 15 – **Scheme of a large river watershed** (after Ciruna and Braun, 2005)

The importance of these environments in terms of diversity in addition to the factors threatening them, rank freshwater environments as priority for conservation. In this perspective, acquiring knowledge about their ecological functioning is crucial.

Freshwater ecology

The three different categories of freshwater ecosystems defined above are all associated to watersheds. A watershed is an area of land collecting precipitation and draining it to a common outlet along a freshwater body (Ciruna and Braun, 2005, Figure 15). Watersheds vary in size and a small watershed can be included in a lager one. The boundaries of a watershed are determined by lines of highest altitude (Figure 15). Watersheds are shaped by global processes including climate, vegetation cover and geology. Climate influences the amount of water coming in and out of the watershed via the intensity and abundance of precipitation and evaporation. Vegetation mostly retains water resulting in a slower water flow and influences the water chemistry by bringing organic matter to the water. Finally, several elements of the geology influence freshwater environment: geology influences the slope and thus the water flow, the nature of the ground influences water chemistry and the size of the sediments influences the speed of the flow and the nature and complexity of the habitats in the water. The interactions of those processes result in ecological characteristics of freshwater environments that can be classified in five main ecological factors:

Hydrologic regime Pattern of variation of the volume and/or flow of water (*e.g.* flow, water level, soil moisture)

Water chemistry regime Variation of water chemical composition over time (*e.g.* salinity, pH, alkalinity, hardness, turbidity)

Physical habitat conditions Nature and characteristics of the substrate (*e.g.* geomorphology, sediments/soil nature, organic debris content and size)

Connectivity Spatial arrangement of different water bodies across the landscape and physical connection between them in relation to how it allows or prevents the dispersion of organisms (*e.g.* landscape connectivity, river lateral connectivity)

Biological composition and interactions Biological composition and structure, biological processes characterising the environment (*e.g.* energy regimes and fluxes, predation, reproduction, biological diversity)

Freshwater organisms evolved in specific ecological conditions, including the variations in water flow, water chemistry and substrate. Human activities may modify one of these key ecological factors or more which in turn will change the overall functioning of the environment.

For example in the river Rhône, infrastructures such as dams for hydropower or embankments to contain the potential flooding events have been built since the 18^{th} century. These infrastructures have had tremendous impacts on flow regimes in the river and its surrounding environments. Dams have reduced minimum water discharge of some portions of several orders of magnitude (*e.g.* 1000 $m^3.s^{-1}$ to 10 $m^3.s^{-1}$ in Lyon, France; Lamouroux et al., 2015). This reduction of flow has particularly impacted secondary channels which are shaped by flooding events (Castella et al., 2015). On the other hand, embankments limit the progressive expansion of the river when there is a flood. The expansion of the river bed during floods results in the water being retained longer and therefore flowing slower which reduces the impact of floods. The embankments help contain small floods locally, however they prevent the expansion of the river upstream resulting in worse floods downstream. It is thought that most of the serious consequences of the flood in the lower Rhône river in 2003 can partly be attributed to human constructions. Therefore the modification of flow regimes can result in deleterious consequences for human activities as well as biological diversity.

Conserving freshwater environments and their species first consists in keeping or restoring the key ecological factors in "natural" conditions (Silk and Ciruna, 2005). It is therefore necessary to establish what these natural conditions are for key ecological factors, and to be able to monitor deviations from these conditions (Hawkins and Norris, 2000). Depending on the financial and time resources, and the knowledge, more or less accurate indicators will be afforded. For example, to assess the quality of the water, chemical sensors can be used to measure the concentration of several pollutants, this requires either precise field sensors or a collection water samples to analyse them in the laboratory. Most of the time, to assess the water quality in biodiversity studies, macro-invertebrate communities are sampled and analysed. Indeed several taxonomic groups such as Plecoptera or Trichoptera are highly sensitive to pollution and are therefore good indicators of water quality (Oertli et al., 2005).

Classical method for key ecological factors monitoring include chemical and physical sensors, and sediment, water, or species collection (Braun, 2005). As explained in the section *Sampling methods in ecoacoustics: Passive Acoustic Monitoring*, these methods suffer a few caveats which could be solved by PAM. This is especially true in freshwater environments where collecting biological samples is an invasive process which does not always allow high temporal and spatial replication. Acoustic monitoring has the potential to solve these two main issues of classical sampling methods however it is unknown to what extend PAM could represent an interesting option for freshwater environment. Indeed, the level of knowledge on freshwater acoustics appears to be relatively low.

Current knowledge in freshwater acoustics

The emission of sounds underwater is known since the 4th century BC as reported for fish in ”Historia animalia” by Aristotle (Book IV, Chapter 4). However, the overall level of knowledge on freshwater sound producing species is relatively small. In freshwater, there are four main taxonomic groups known for producing sounds: amphibians, actinopterygian, crustaceans and insects (Figure 16). Although these four groups include a substantial diversity of species and sounds, there are no reference cataloguing these sounds.

Freshwater soundscapes of lakes and rivers have been poorly investigated. When investigating the geophonic component of freshwater soundscapes, Tonolla et al. (2010) distinguished pools, riffles, runs with and without stream-bed sediment transport, and step-pools, four main types of riverine habitats, thanks to the amplitude in the octave bands of those recorded habitats. Other studies investigated the variability of ambient noise in various habitats (Wysocki et al., 2007) and year round (Amoser and Ladich, 2010). Additionally, another study explored the antropophonic component of freshwater environments by studying the soundscapes of lakes across an urbanisation gradient, show-

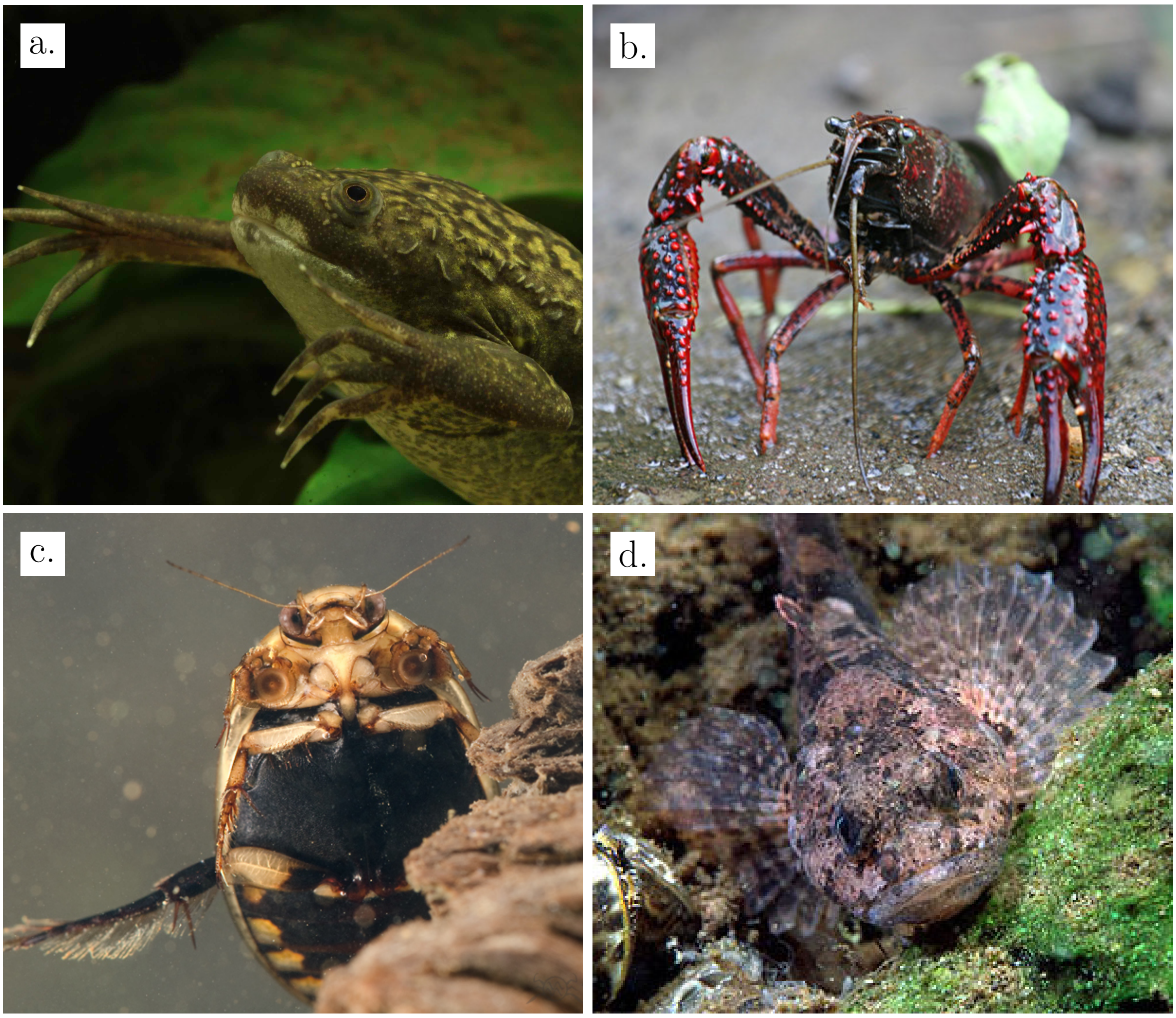

Figure 16 – **Example of sound producing species in freshwater environments**. a. African clawed frog, *Xenopus laevis* (wikimedia), b. Red swamp crayfish, *Procambarus clarkii* (wikimedia), c. A species of water beetle, *Acilius sulcatus* (picture: Jan Hamrsky), d. Common bullhead, *Cottus gobio* (wikimedia)

ing an increase of the level of anthropophony with increasing urbanisation (Kuehne et al., 2013). These acoustic differences are mainly due to sediment transport and turbulence sounds or anthropogenic sounds respectively. The existence of such acoustic signatures suggests the interest of using acoustics to identify variations in key ecological factors such as hydrological regime or anthropisation.

A few acoustic populations have also been studied such as populations of a fish species, the burbot (*Lota lota*) in Canadian lakes (Cott et al., 2014). To my knowledge, no acoustic population of amphibian or aquatic insects has been studied underwater although several acoustic populations of amphibians have already been studied thanks to aerial recordings (Llusia et al., 2013b). Finally, no freshwater acoustic community has ever been studied. The current knowledge on freshwater acoustics therefore appears relatively low, especially when considering the nature of biological acoustic sources and the composition and structure of acoustic diversity.

Aims and outline

Gaps in our knowledge

Freshwater environments are extremely threatened by human activities. The protection of threatened environments requires a deep knowledge on its functioning and good indicators to monitor the impacts of human activities or restoration.

Several freshwater animals emit sounds underwater. Until now, bioacoustics in freshwater environments mainly focused on amphibians and some fishes (Llusia et al., 2013b; Cott et al., 2014). As Jérôme told me when I first started to get interested in the project of this PhD: ”Freshwater acoustics is as explored as bird songs were in the fifties: we know virtually nothing ”. This convinced me of the interest but also the heaviness of the task.

In recent years, technical progress has enabled the development of PAM. This monitoring tool has a great potential for many environments including freshwaters. PAM has never been applied to freshwater environments. We therefore wonder whether PAM could also be applied in freshwater environments.

One necessary step to conducting PAM in an environment is to test the link between acoustics and environmental conditions. Needless to say that if freshwater sound producers have poorly been studied, the link between acoustics and environmental conditions is even less explored. As pointed out in the section *Ecoacoustics: Linking acoustics and ecology*, ecoacoustics is a recently emerged discipline which is currently under development. One of the aims of ecoacoustics is to develop knowledge on the factors structuring soundscapes, acoustic communities and populations. Freshwater acoustics have been lagging behind many other environments although small habitats such as ponds and secondary channels appear as interesting model systems to establish this emerging discipline (Pereira et al., 2011).

General problematics

The general aim of this thesis is to investigate the existence of a link between acoustic and ecology in freshwater environments. This link will be explored in several freshwater environments at different spatial and ecological scales. The study of freshwater environments will be undertaken in France, where the biological diversity and ecology has already been studied by a large community of freshwater specialists.

Outline

What are the acoustic sources in freshwater environments ? Knowledge about species emitting sounds exists but has never been reviewed across taxa. The amount of diversity that can be expected in freshwater environments is the subject of **Chapter 1** which reviews the literature on sound production in freshwater organisms and is completed by my own laboratory recordings. This chapter highlights a significant diversity of sound emitters in France as well as the generally low level of knowledge on the sounds produced.

Freshwater environments are potentially inhabited by a rich variety of sound emitters, however, is this diversity translated in acoustic communities and how ? **Chapter 2** investigates for the first time the diversity and structure of acoustic communities in three freshwater ponds. This chapter highlights a previously unexplored acoustic diversity found in freshwater environments and reveals that this diversity, rather than being randomly distributed, is structured both spatially and temporally.

Freshwater acoustic communities reveal spatio-temporal structures, what are the environmental factors linked to these acoustic assemblages ? **Chapter 3** and **Chapter 4** explore the effects of several environmental variables on acoustic communities and populations to investigate the existence of a link between acoustics and ecology in freshwater environments and infer the potential ecological processes shaping acoustic communities.

In **Chapter 3**, we explore the environmental factors structuring acoustic communities in six secondary channels of the river Rhône. We highlight the importance of one ecological variable, the lateral connectivity, on the acoustic community composition.

In **Chapter 4**, we test the effect of noise on an acoustic population of *Micronecta scholtzi* (Insecta, Hemiptera, Corixidae) with an acoustic monitoring and a noise playback experiment. The monitoring revealed a periodicity of the acoustic activity of the population of *Micronecta scholtzi*. This acoustic activity was linked negatively to the presence of vegetation and the temperature. Finally, the playback experiment revealed the positive relationship between anthropogenic noise and the level of acoustic activity of a population of aquatic insects.

Finally, **Chapter 5** puts the results of this PhD in a more general background and sketches the future directions opened by this work.

The first four chapters are introduced by an illustrated abstract summarising the context of the study, the problematics, the methods used, the main results and some perspectives.

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Chapter 1 :

Who sings underwater ? A review of sound emitters found in French freshwater environments

Ponds of the Mine de Cap Garonne, Le Pradet (Var, 83), May 2013

1.1 Summary

Context Some freshwater species are known to produce sounds underwater. Using passive acoustic monitoring thus appears as an interesting complementary approach to classical sampling methods in freshwater environments. However knowledge on sound producing species has never been summarised across taxa.

Problematics

- What is known about sound production in freshwater environments in the literature ?
- What are species specific sound productions of freshwater insects and crustaceans ?

Methods

- Literature review
- Recording of insect and crustacean species isolated in an aquarium under controlled laboratory conditions

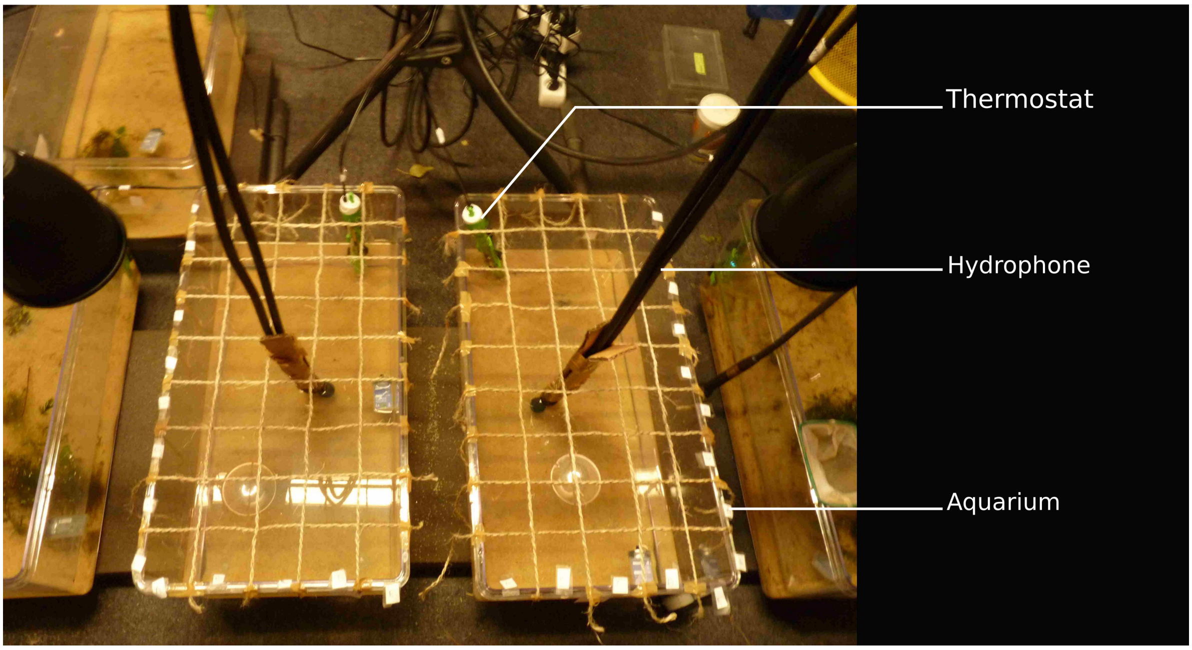

Figure 1.1 – **Two recording set ups in the acoustic chamber of the laboratory**. Each set up consists in an aquarium containing the isolated species and a hydrophone plugged to a recorder.

Main results Extensive literature review reveals that at least 271 species produce sounds underwater in French freshwater environments. The knowledge on sound production by freshwater organism is relatively scattered: the sound characteristics (*e.g.* frequency, duration) of only 35 % of the soniferous species have been described formally in the literature.

Among the 19 species recorded in our laboratory, eight different species produced sounds and among them, two had never been reported in the literature. However, recording species in the laboratory proved less successful than expected as 94 % of the recording time was without any sound production. Moreover three species, known to be soniferous, never produced any sounds during our recordings.

Perspectives A better knowledge on sound production by freshwater organisms may enable species identification and monitoring with sounds. It is necessary to join forces with other laboratories to identify species specific sounds and mutualise our common knowledge in sound repositories. Exploring the possibility of recording individuals directly in the field or in more naturalised aquarium may help to identify species more successfully.

Related communications and publications

- Acoustic methods in freshwater systems: A new frontier in continuous system monitoring. Simon Linke, **Camille Desjonquères**, Toby Gifford. Special section accepted in *Freshwater Biology.* (Appendix 6.1.1).
- Real-time Ecosystem Monitoring using Passive Acoustics in Freshwater Environments. Simon Linke, Toby Gifford, **Camille Desjonquères**, Diego Tonolla, Leah Barclay, Fanny Rybak, Jérôme Sueur, Chris Karaconstantis, Mark Kennard, Thierry Aubin, Doug Demko. Manuscript in preparation to be submitted in *Frontiers in Ecology and the Environment.* (Appendix 6.1.2).
- Exploration acoustique des milieux d'eau douce : Une nouvelle approche du suivi de la biodiversité. **Camille Desjonquères**. 2014. Invited oral communication at the Journée Fleuves et Rivières, Muséum national d'Histoire naturelle, Paris.

1.2 Introduction

In freshwater, biodiversity is declining more rapidly than in any other realm (Dudgeon et al., 2006). Freshwater environments include a diversity of water bodies ranging from small ones, such as ponds or streams, to large ones, such as lakes or rivers. These environments are threatened by several anthropological activities such as drainage, pollution (agricultural fertilization or industrial waste), cattle paddling or dam construction (Wood et al., 2003; Dudgeon et al., 2006). These activities result in direct loss of habitats, fragmentation of the network of freshwater habitats (pondscape or riverscape, Wood et al., 2003) and habitat quality alteration (*e.g.* pollution or desiccation). Yet these water bodies have a lot to offer. European freshwater habitats host a large number of endemic species, some of which are highly threatened (Céréghino et al., 2008; Keith et al., 2011). A hotspot of diversity as defined by Myers et al. (2000) is characterized by two main features namely, the "number of endemic species" and the "degree of threat". Following this definition, some authors have categorised freshwater habitats as hotspots for animal diversity in temperate climate (Myers et al., 2000; Verberk et al., 2006). These environmental changes highlight the need for efficient monitoring methods to help ranking conservation priorities.

Traditionally, monitoring animal presence and diversity in freshwater systems is conducted using techniques such as netting and electro-fishing. These techniques however have a number of major drawbacks: i) they potentially injure the organism and in the case of macro-invertebrate even result in death, ii) they bias the outcomes by capturing only the individuals or species which do not succeed in escaping, iii) they require a lot of workforce and time, iv) they are most of the time conducted with low spatial and temporal replication. The last point especially is a key flaw in traditional techniques – continuous monitoring of aquatic systems is imperative to resolve complex spatio-temporal interplay in riverine processes (Goodman et al., 2015).

The use of acoustic recordings as an un-invasive method to sample and monitor ecosystems has been suggested in several articles (Furnas and Callas, 2015; Heinicke et al., 2015; Felisberto et al., 2015; Blumstein et al., 2011). This method, currently in development, consists in recording the sounds of an environment, either in air or in water to extract information such as ecosystem health and dynamics (Sueur et al., 2008; Fuller et al., 2015; Pieretti et al., 2011), or presence of specific species (Ulloa et al., 2016). Although it has rarely been applied yet, passive acoustic monitoring of freshwater environments is a method which has the potential to circumvent the issues with traditional sampling techniques. Acoustic monitoring of aquatic systems is a un-invasive sampling method that requires the introduction of a hydrophone underwater in the habitat to record the sounds emanating from the environment. With this method, sounds produced underwater and at air/water interface (such as a frog calling in the water) are recorded. Doing so has no effect on the individuals' health. Detection does not frighten the individuals and therefore is not biased towards slower individuals. With the advent of inexpensive autonomous and weather resistant recorders, acoustic monitoring can be undertaken without too much efforts and costs. Finally spatial and temporal repetition can be easily obtained by placing several hydrophones and setting a proper recording schedule.

Passive acoustics has the potential to enable real time monitoring of species producing sounds. Yet, to evaluate how efficient it could be and to which species it can apply, summarizing the current knowledge on acoustic production by freshwater organisms is crucially needed. Up to now, the knowledge on sound production by freshwater organisms has never been summarised across taxa. We reviewed the literature on sound production of freshwater organisms found in France. We first summarise the knowledge on acoustic production in the main groups known to produce sounds. We then underline the taxonomic groups which require further study. Finally we explore some of the species requiring further study with laboratory recordings.

1.3 What do we already know ?

This section is a review of the existing literature on acoustic production of aquatic animals. For each of the four taxa in which sound producing species are known the following points will be examined: i) Species and groups known to produce sounds, ii) behavioural contexts of emission, iii) mechanisms of emission, and iv) characteristics of the emitted sounds.

1.3.1 Amphibians

There are 62 Amphibian species in Europe, among them 35 are Anura, 27 are Caudata and none are Gymnophiona (Team, 2014). We will consider only the species which spend a significant amount of their adult lifetime in freshwater. Anurans produce a high diversity of aerial and aquatic acoustic signals during both male-male and female-male interactions (Ryan, 2001). Among the 28 species found in France, only one Anuran, *Alytes obstetricans*, does not have an aquatic adult stage. Therefore up to 27 Anuran species can be recorded underwater in French freshwater environments.

The sounds are mostly emitted by males during choruses involved in male-male and female-male interactions in the context of reproduction (Ryan, 2001). The males emit loud calls to which the females reply by displaying phonotaxis (*i.e.* orientation and displacement towards the sound source, Ryan, 2001). Females also produce less conspicuous calls which have been less studied. For example, in *Bombina variegata*, if the male has an inappropriate position during mating, the female will call until the male seizes her properly (Duguet and Melki, 2003). A few other signals have been described such as the distress calls or alarm calls (Gerhardt and Huber, 2002).

The sound production mechanism consists in forcing air from lungs to the vocal sac through the larynx (Figure 1.2), back and forth in the case of *Discoglossus* (Gerhardt and Huber, 2002) or from the oral cavity to the lungs in the case of *Bombina variegata*. The air flow induces the vocal cords to vibrate. The larynx contains muscles controlling the elongation of the vocal cords. In most cases, the sound produced by the vibration of the vocal cords is then amplified by the vocal sac. Some species do not possess a vocal sac (Table 1.1), such as *Bombina variegata* or *Xenopus laevis*. However only some member of the family of *Xenopus laevis* (Pipidae) differ in their sound production mechanism. In *Xenopus laevis*, the larynx lacks vocal cords and constitutes a large and ossified box (Irisarri et al., 2011). This box contains the arytenoid cartilages which are modified into two bony rods. The sound production is mediated by the contraction of laryngeal muscles which induce implosion of air into a vacuum formed by rapidly moving disk-like enlargements of these two rods (Irisarri et al., 2011). The pulse is then amplified by the large vocal box.

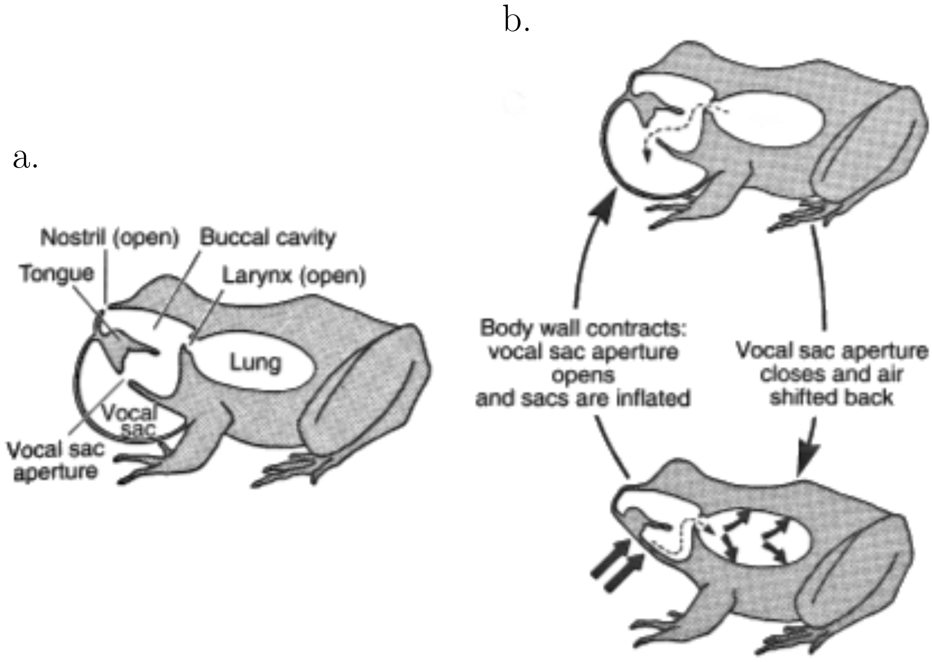

Figure 1.2 – **Anuran sound production mechanism** (after Gerhardt and Huber, 2002, Figure 2.7, page 23). a. Anatomy of the vocal tract of Anurans, b. Air flow during sound production in Anurans.

Most species produce sounds in air or at the water/air interface. In European species, calls are usually below 7 kHz (see Table 1.1) with a relatively low to no frequency modulation (Gerhardt and Huber, 2002). The usual temporal structure of Anuran calls consists in a repetition of notes (Figure 1.3). One note is usually defined as the sound unit produced by one expiratory event (Gerhardt and Huber, 2002). These notes vary in timing but commonly last a few seconds (Gerhardt and Huber, 2002).

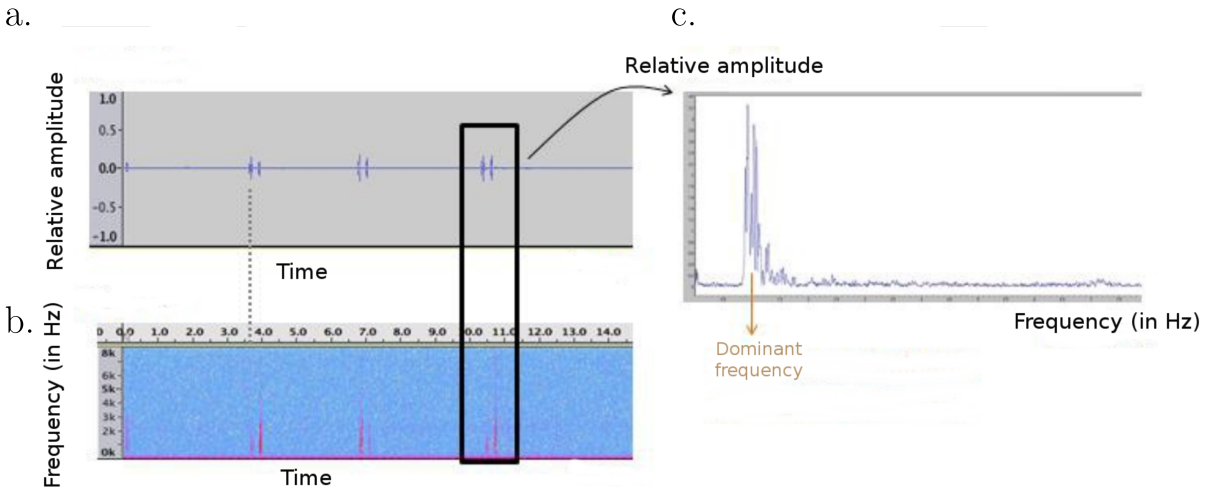

Figure 1.3 – **Underwater sound production of *Pelobates fuscus*** (after Dutilleux and Curé, 2016). a. Oscillogram, b. Spectrogram, c. Power spectrum of the selection shown in a. and b.

Table 1.1 – **Sound production in Anurans**. Mechanism: 1: air through larynx amplified by the vocal sac, 2: air through larynx, 3: air implosion due to laryngeal muscle contraction (Duguet and Melki, 2003).

Species	**Sex**	**Mechanism**	**Frequency range (dominant frequency) in Hz**	**Behavioural context**
Discoglossus montalentii	Male	1	200-1500 (600)	Reproduction
Discoglossus pictus	Male	1	900-2200 (1100)	Reproduction
Discoglossus sardus	Male	1	500-2000 (850)	Reproduction
Bombina variegata	Male	2	500-3000 (550)	Reproduction
Bufo bufo	Male	1	200-5500 (1100)	Reproduction
Bufo spinosus	Male	1	200-5500 (1100)	Reproduction
Bufo calamita	Male	1	200-5000 (1700)	Reproduction
Pseudepidalea balearica	Male	1	900-2700 (1300)	Reproduction
Pseudepidalea viridis	Male	1	1200-4500 (1450)	Reproduction
Hyla arborea	Male	1	1000-5500 (2650)	Reproduction
Hyla meridionalis	Male	1	650-3500 (800)	Reproduction
Hyla sarda	Male	1	1000-6000 (2400)	Reproduction
Pelobates cultripes	Male	1	200-1200 (550)	Reproduction, distress
Pelobates fuscus	Male	1	200-2100 (450)	Reproduction, distress
Pelodytes punctatus	Male	1	500-4500 (1050)	Reproduction
Xenopus laevis	Both	3	1200-5500 (1600)	Reproduction
Pelophylax bergeri	Male	1	500-3500 (2150)	Reproduction
Pelophylax esculentus	Male	1	400-5500 (2300)	Reproduction
Pelophylax grafi	Male	1	300-7000 (1750)	Reproduction
Pelophylax lessonae	Male	1	300-6500 (1850)	Reproduction
Pelophylax perezi	Male	1	600-6500 (1850)	Reproduction
Pelophylax ridibundus	Male	1	250-6000 (1800)	Reproduction
Rana arvalis	Male	1	300-1700 (450)	Reproduction
Lithobates catesbeiana	Male	1	150-5000 (1100)	Reproduction
Rana dalmatina	Male	1	100-3500 (550)	Reproduction
Rana pyrenaica	Male	1	100-3500 (800)	Reproduction
Rana temporaria	Male	1	200-1900 (400)	Reproduction

1.3.2 Fish (Actinopterigii)

Out of the 108 species of fish included in two infra-class (telostei and chondrostei) and 25 families in freshwater habitats in France (Keith et al., 2011; Denys et al., 2014), five are known to produce sound so far (Table 1.2). These five species include two species of stream gobies (*Gobius paganellus* and *Gobius cobitis*, Lugli et al., 2003), two species of river bullheads (*Cottus rhenanus* and *Cottus perifretum*, Colleye et al., 2013) and the burbot (*Lota lota*, Cott et al., 2014).

Sounds have been reported in the context of reproduction and territory defence. In Gobies, the sounds are emitted by males during courtship and mating interactions (Lugli, 2010). In river bullheads, sounds are emitted both by males and females in agonistic and courtship contexts (Colleye et al., 2013). In the burbot, the calls are emitted coincidently with the start of their spawning period (Cott et al., 2014).

In river bullheads, the sounds are produced by vibration of the pectoral girdle (Colleye et al., 2013, , Figure 1.4b). In burbots, the presence of a swimbladder and the similarity of their sounds with those produced with the swimbladder of cods - a closely related species - suggests an identical mechanism of sound production (Cott et al., 2014, , Figure 1.4a). In Gobies, sound production does not seem to involve contraction of the swimbladder as in many species of fishes. Indeed the deflation of the swimbladder in *Padogobius martensii* or the absence of swimbladder in *Gobius nigricans* did not affect their ability to produce sounds (Lugli et al., 2003). The current hypothesis is that they produce sound with sonic muscles but the sound is not amplified by the swimbladder (Lugli, 2010).

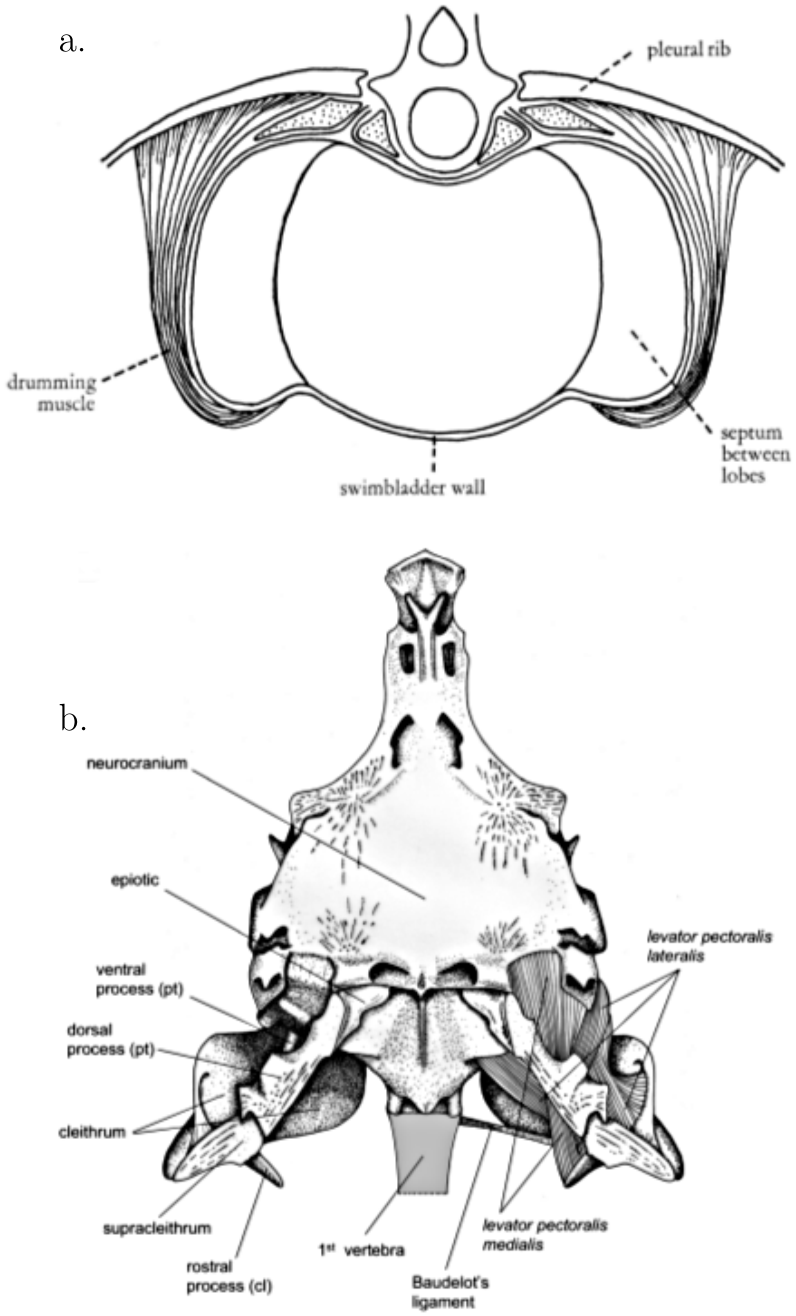

Figure 1.4 – **Two sound production organs in fish**. a. Transverse section of the thorax of a cod (*Gadus morhua*) at the level of the drumming muscles showing the swimbladder. The drumming muscle contract on the swimbladder to emit the sounds (after Brawn, 1961), b. Morphology of the neurocranium and pectoral girdle in *Cottus perifretum*. Dorsal view of the skeletal elements of the pectoral girdle (left) and the different muscles involved in pectoral girdle adduction (right). pt, post-temporal; cl, cleithrum (after Colleye et al., 2013).

Table 1.2 – **Sound production in fishes**

Order	**Family**	**Species**	**Sex**	**Mechanism**	**Frequency range (dominant frequency) in Hz**	**Behavioural context**	**Reference**
Perciformes	Gobiidae	*Gobius paganellus*	male	?	69–98 (?)	Courtship	Lugli et al. 2003
Perciformes	Gobiidae	*Gobius cobitis*	male	?	46–56 (?)	Courtship	Lugli et al. 2003
Scorpaeniformes	Cottidae	*Cottus rhenanus*	both	Pectoral girdle vibration	110-237 (170)	Courtship, territory defense	Colleye et al. 2013
Scorpaeniformes	Cottidae	*Cottus perifretum*	both	Pectoral girdle vibration	65-194 (108)	Courtship, territory defense	Colleye et al. 2013
Gadiformes	Lotidae	*Lota lota*	?	Swimbladder	50-500 (100)	Reproduction	Cott et al. 2014

The sounds emitted range from 50 to 500 Hz (Table 1.2). They consist mainly in grunts or hums and pulses. Pulses usually last about a few hundred milliseconds. Grunts are usually low in frequency with low to no frequency modulation and vary in duration from a few seconds to a little less than a minute (Figure 1.5).

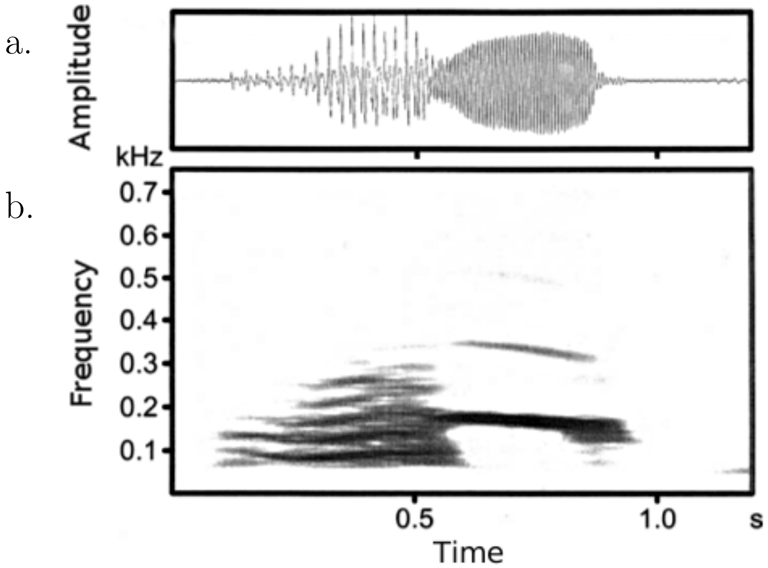

Figure 1.5 – **Sound production of *Padogobius martensii*, an Italian Gobiidae**. Presentation of a complex sound (a pulse-train sound followed by the tonal sound; after Lugli et al., 2003), a. Oscillogram (amplitude: 100 mV/division, arbitrary units), b. Spectrogram.

1.3.3 Crustaceans

Three classes of Crustaceans are found in freshwater: Branchiura, Branchiopoda and Malacostraca. The Louisiana crayfish (*Procambarus clarkii*), an invasive species found in France, is the only Crustacean which has been yet reported for sound production (Favaro et al., 2011).

The sounds, emitted by both males and females, were recorded in air in the laboratory and were related to up and down movements of the scaphognathite - the appendage driving the water flow to the gill cavity (Figure 1.6).

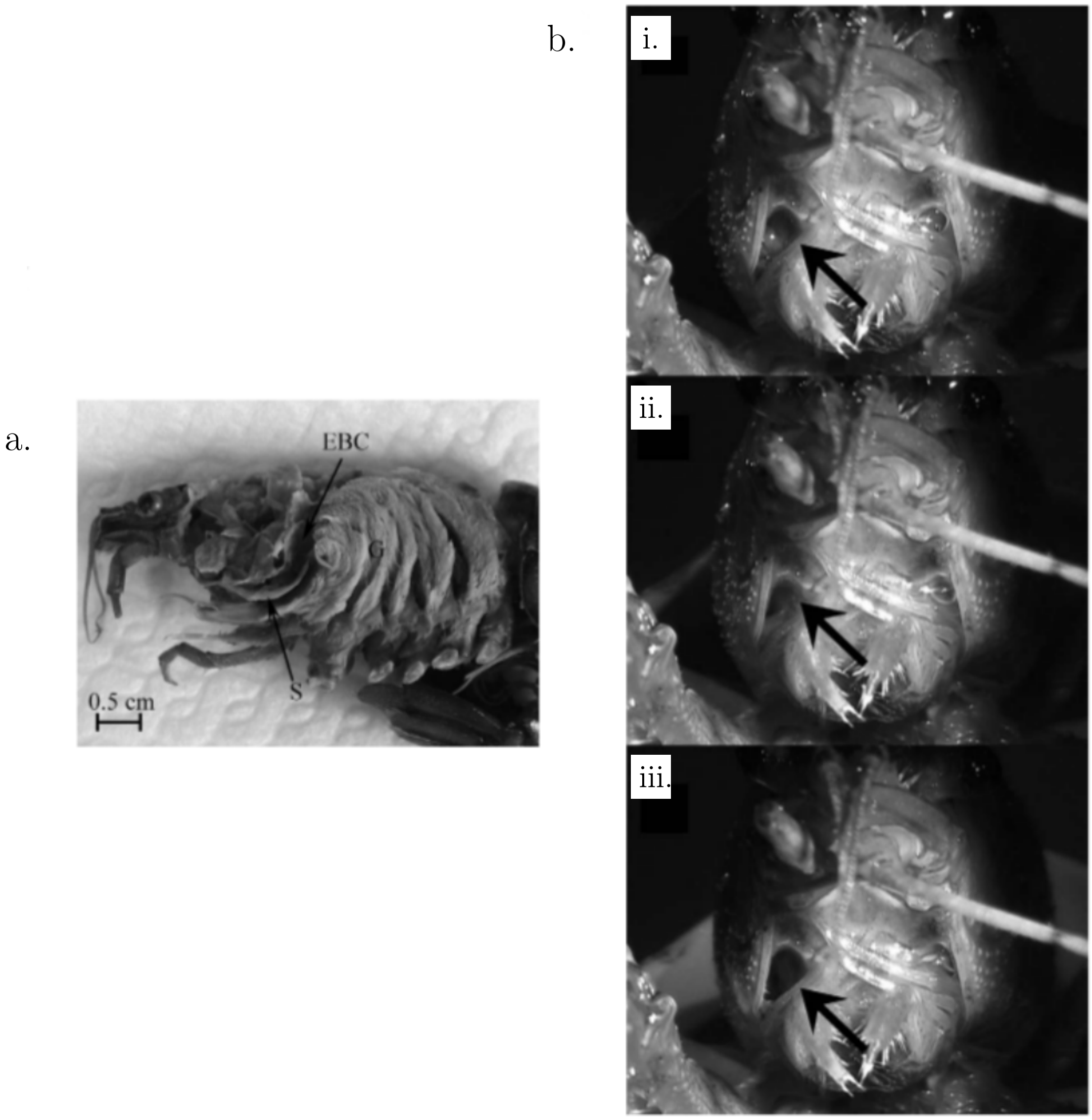

Figure 1.6 – ***Procambarus clarkii* sound production organ** (after Favaro et al., 2011). a. Lateral view of the anatomy of the thoracic region of *Procambarus clarkii* showing the gills (G), the efferent branchial channel (EBC) and the scaphognathite (S) b. Frontal view of a specimen of *Procambarus clarkii* showing a complete scaphognathite waving: i. closed efferent branchial channel (arrow), ii. half open efferent branchial channel (arrow) and iii. wide open efferent branchial channel (arrow).

No production was found when the scaphognathite was experimentally ablated. However the producing organ and mechanisms of production have not been precisely described yet.

The sounds are very short sounds units (pulses) produced in trains. Within individuals, the number of pulses per train can vary, as well as the pulse repetition rate (Favaro et al., 2011). The dominant frequency and duration of the pulses has not been measured precisely yet (Figure 1.7).

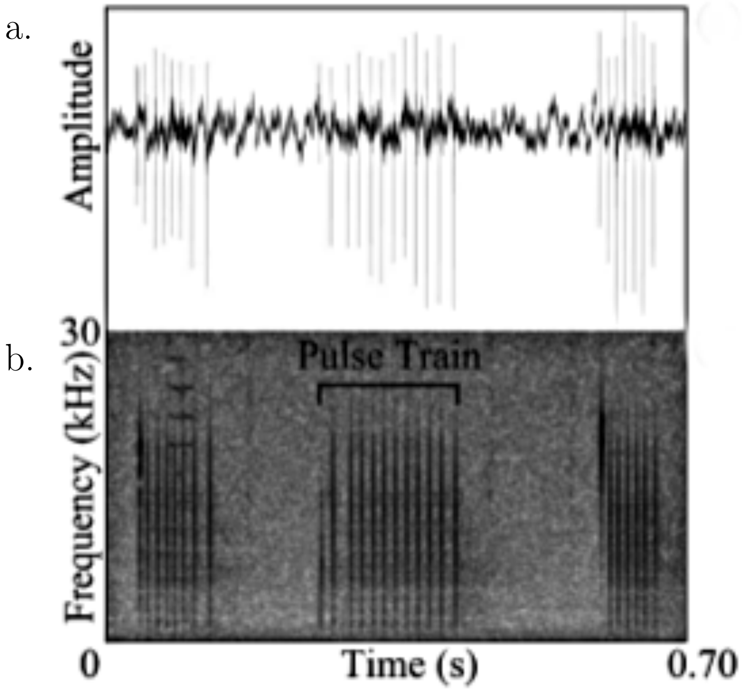

Figure 1.7 – **A pulse train produced by a *Procambarus clarkii* specimen at 5 cm from the microphone** (after Favaro et al., 2011). The sound is shown as a. oscillogram and b. spectrogram.

1.3.4 Insects

Aquatic insects are defined as insects which have at least one aquatic life stage (larvae/juvenile, adult or both). French aquatic insects are distributed among 11 orders (Tachet et al., 2000). The first note on sound production in aquatic insects was published by Frisch (1720) for a creeping water bug (*Ilyocoris cimicoides*, Hemiptera) who describes a violin sound when handling this insect. Later, a lot of the work of describing sound production in aquatic insects, especially aquatic bugs such as Corixidae, was done by Jansson in the 70's and 80's. In total, up to now, the production of sounds is known in 36 genera belonging to three orders: 21 genera of Coleoptera, 14 genera of Hemiptera and one genus of Trichoptera (Table 1.3). Knowledge at the species level is not always available in the literature, or not easy to find as a consequence of the changes in the taxonomic classification of the groups. We therefore consider genus level for insects. In the articles in which species are clearly spelled out (Jansson, 1989), it is common that all the species within one genus produce sound. It is therefore reasonable to assume that all the species in the 36 insect genera known to produce sounds indeed emit sound. According to the number of species in those genera, we expect 238 species of aquatic insects to emit sounds in freshwater environments in France.

The sounds are emitted mostly by males in a reproduction context, but the precise behavioural sequences are still poorly documented (Aiken, 1985). In *Palmacorixa nana* (Hemiptera), males have been shown to aggregate (Aiken, 1982a) and females to spend more time in places with higher levels of acoustic activity. However, several studies have been unable to demonstrate an actual phonotaxis of females (Aiken, 1982a; Jansson, 1973). In several species, adults (*Hygrobia hernani*, Coleoptera, Aiken, 1985) or juveniles (*Hydrophilus piceus*, Coleoptera Aiken, 1985), produce sounds in a distress context, when handled for example. Other less common behavioural contexts have been documented such as mass dispersion in *Eretes sticticus* (Coleoptera, Kingsley, 1985), territory defense in Hydropsychid larvae (Jansson and Vuoristo, 1979). Lastly, before emerging from the pond and flying away, *Acilius sulcatus* (Coleoptera) were found to produce low frequency sounds thought to correspond to flight muscle contraction (Leston et al., 1965).

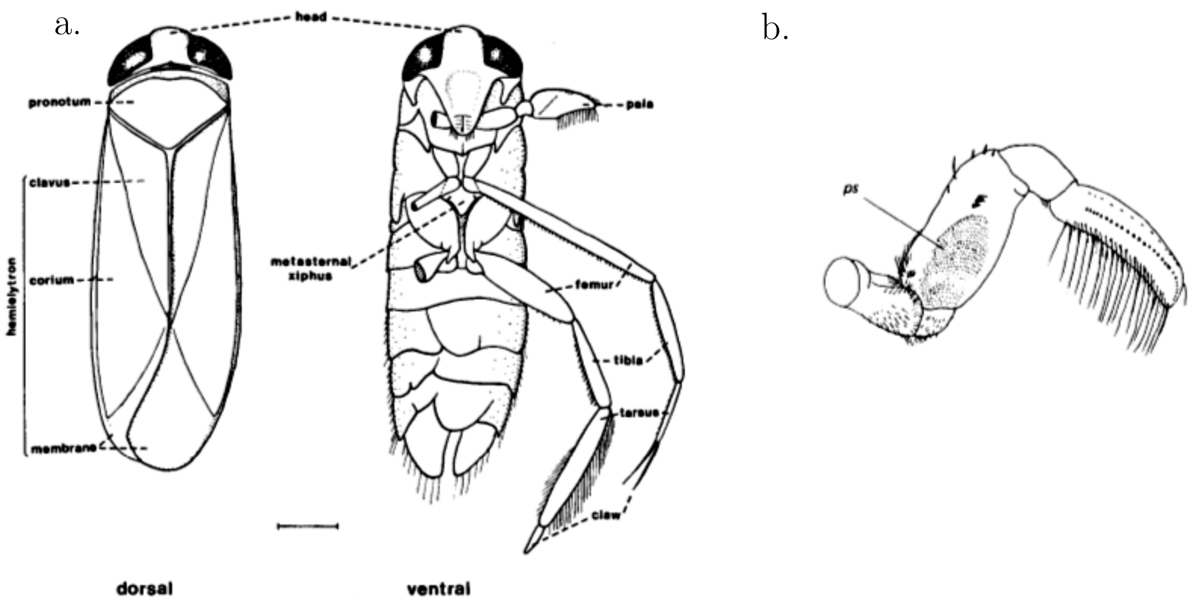

Figure 1.8 – **Corixidae morphology and stridulatory organ**. a. General morphology of a male Corixidae, here *Sigara falleni* (Hemiptera, scale bar: 1 mm). The plectrum is located on the head, along maxilary plate (after Savage, 1990), b. Location of the stridulatory file in a male Corixidae, here *Corixa punctata* (Hemiptera, ps: par stridens, after Aiken, 1985).

In most cases, the described mechanisms to produce sounds consist in a stridulation (Table 1.3). Corixidae and Anisops stridulate by rubbing a file located on their fore legs against a ridge on the side of their head (Figure 1.8, Aiken, 1985). In Hydropsychidae, the file is located on the underside of the head and the plectrum on the fore legs (Johnstone, 1964). In several genera of Coleoptera and in *Plea minutissima* (Hemiptera), the stridulatory organs, either the file or the plectrum and the file, are located on the abdomen (Aiken, 1985). Some other Coleoptera have their stridulatory organs located on the elytra. Micronectidae (Hemiptera) produce sound by rubbing a striated part of their genitalia (located on the right paramere) against a ridge of their abdomen (Jansson, 1989). Apart from stridulation, two other cases have been documented so far: expulsion of air through mesothoracic spiracles in *Cybister confusus* (Coleoptera) larvae (Mukerji, 1929) and muscle contraction in *Acilius sulcatus* (Coleopteran, Leston et al., 1965).

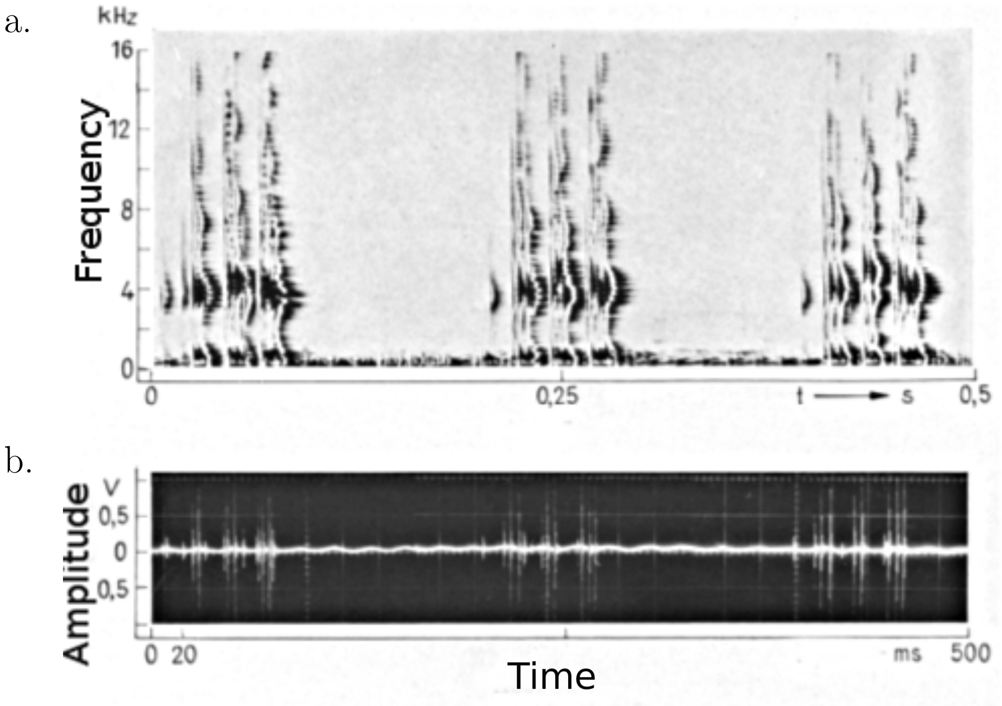

Figure 1.9 – **Sound production of *Callicorixa praeusta* (Hemiptera)**. a. Spectrogram, b. Oscillogram. (after Finke, 1968).

Among the 36 Genera presented in Table 1.3, seven genera, representing a total of 60 species, have a known frequency range of sound production. The frequencies vary from 200 Hz for *Acilius sulcatus* (Coleoptera, Leston et al., 1965) up to 100 kHz for Hydropsychid larvae (Trichoptera, Silver and Halls, 1980). Temporal features of aquatic insect sounds are rarely available, except for a few well studied Corixidae species such as *Corixa dentipes* (Hemiptera) (Theiss, 1983). Owing to their sound production mechanisms, most species produce low modulated broad band thrills consisting of short pulses (Figure 1.9). In some species, the sound production include two different parts with different temporal features such as in *Micronecta minutissima* (Hemiptera Jansson, 1989).

Table 1.3 – **Sound production in aquatic insects**. f: file, s: scraper.

Order	**Familly**	**Genus**	**Life stage**	**Sex**	**Mechanism**	**Frequency range (kHz)**	**Behavioural context**	**Reference**
Coleoptera	Hygrobiidae	*Hygrobia*	Adult	?	f: elytra near the margin, s: tip of the abdomen	?	Distress, defence	Aiken 1985; Balfour-Browne 1922
Coleoptera	Dytiscidae	*Acilius*	Adult	Male	Muscle contraction	?	Preparation for flight	Leston et al. 1965
Coleoptera	Dytiscidae	*Dytiscus*	Adult	?	?	?	Reproduction	Aiken 1985; Blunck 1913
Coleoptera	Dytiscidae	*Eretes*	Adult	?	?	?	Mass migration	Kingsley 1985
Coleoptera	Dytiscidae	*Cybister*	Larva/Adult	?/?	Air expulsion by mesothoracic spiracles/?	?	Defence/?	Mukerji 1929
Coleoptera	Haliplidae	*Haliplus*	Adult	?	f: elytra, s: toothed area on the pleural fold	?	?	Aiken 1985
Coleoptera	Hydrophilidae	*Spercheus*	Adult	?	f: 3rd abdominal segment, s: lower face of the elytron	?	Reproduction	Aiken 1985; Buhk 1910
Coleoptera	Hydrophilidae	*Anacaena*	Adult	?	f: 3rd abdominal segment, s: lower face of the elytron	?	?	Aiken 1985

Order	**Familly**	**Genus**	**Life stage**	**Sex**	**Mechanism**	**Frequency range (kHz)**	**Behavioural context**	**Reference**
Coleoptera	Hydrophilidae	*Berosus*	Adult	Female	f: 3rd abdominal segment, s: lower face of the elytron	?	Reproduction	Tassell 1965
Coleoptera	Hydrophilidae	*Enochrus*	Adult	?	f: 3rd abdominal segment, s: lower face of the elytron	?	Reproduction	Adams and Miller 1980
Coleoptera	Hydrophilidae	*Helophorus*	Adult	?	f: 3rd abdominal segment, s: lower face of the elytron	?	?	Aiken 1985
Coleoptera	Hydrophilidae	*Hydrobius*	Adult	?	f: 3rd abdominal segment, s: lower face of the elytron	?	Defense, reproduction	Aiken 1985
Coleoptera	Hydrophilidae	*Hydrochara*	Adult	?	f: 3rd abdominal segment, s: lower face of the elytron	?	?	Aiken 1985
Coleoptera	Hydrophilidae	*Hydrochus*	Adult	?	f: 3rd abdominal segment, s: lower face of the elytron	?	?	Aiken 1985
Coleoptera	Hydrophilidae	*Hydrophilus*	Adult	?	f: 3rd abdominal segment, s: lower face of the elytron	?	?	Meyer-Rochow 1971

Order	**Familly**	**Genus**	**Life stage**	**Sex**	**Mechanism**	**Frequency range (kHz)**	**Behavioural context**	**Reference**
Coleoptera	Hydrophilidae	*Laccobius*	Adult	?	f: 3rd abdominal segment, s: lower face of the elytron	3-5	?	Pirisinu et al. 1988
Coleoptera	Hydrophilidae	*Limnoxenus*	Adult	?	f: 3rd abdominal segment, s: lower face of the elytron	?	?	Meyer-Rochow 1971
Coleoptera	Hydrophilidae	*Paracymus*	Adult	?	f: 3rd abdominal segment, s: lower face of the elytron	?	?	Aiken 1985
Coleoptera	Hydraenidae	*Hydraena*	Adult	Male	f: head, s: pronotum	?	?	Aiken 1985; Perkins 1980
Hemiptera	Corixidae	*Micronecta*	Adult	Male	f: right paramere, s: ridge of the 8th abdominal segment	5-15	Reproduction	Jansson 1989; Mitis 1935
Hemiptera	Corixidae	*Cymatia*	Adult	?	f: pegs on the fore tarsa, s: opposite femur	?	?	Poisson 1935; Butler 1923
Hemiptera	Corixidae	*Glaenocorisa*	Adult	?	?	?	?	Aiken 1985
Hemiptera	Corixidae	*Corixa*	Adult	Both	f: pegs on the fore femur, s: maxlary plate	1,5-3	Reproduction	Aiken 1985; Theiss 1983

Order	**Familly**	**Genus**	**Life stage**	**Sex**	**Mechanism**	**Frequency range (kHz)**	**Behavioural context**	**Reference**
Hemiptera	Corixidae	*Hesperocorixa*	Adult	?	f: peg on the fore femur, s: Head	?	?	Poisson 1957
Hemiptera	Corixidae	*Heliocorisa*	Adult	?	f: pegs on the fore femur, s: maxlary plate	?	?	Poisson 1957
Hemiptera	Corixidae	*Arctocorisa*	Adult	Both	f: pegs on the fore femur, s: Head	3-4.5	Reproduction	Aiken 1985; Jansson 1979b
Hemiptera	Corixidae	*Sigara*	Adult	Both	f: pegs on the fore femur, s: Head	2.5-5.5	Reproduction	Aiken 1985; Jansson 1979a
Hemiptera	Corixidae	*Callicorixa*	Adult	Male	f: pegs on the fore femur, s: Head	3-6	Reproduction	Finke 1968
Hemiptera	Pleidae	*Plea*	Adult	?	f: mesothorax, s: prothorax	?	group cohesion	Wefelscheid 1912
Hemiptera	Notonectidae	*Anisops*	Adult	Male	f: tibial stridulatory comb, s: ?	?	?	Poisson 1957
Hemiptera	Naucoridae	*Naucoris*	Adult	Male	sclerotized ridge on the 2nd abdominal tergite	?	?	Frisch 1720; Hofeneder 1937
Hemiptera	Nepidae	*Nepa*	Adult	?	Pronotum mesonotum	?	?	Swinton 1877
Hemiptera	Nepidae	*Ranatra*	Adult	?	f: fore coxa, s: fore femur	?	?	De La Torre Bueno 1903

Order	Familly	Genus	Life stage	Sex	Mechanism	Frequency range (kHz)	Behavioural context	Reference
Trichoptera	Hydropsychidae	*Hydropsyche*	Larva	Both	f: underside of the head, s: fore femur	64-100	Defense	Johnstone 1964; Silver and Halls 1980

1.4 Which taxonomic groups should be investigated first ?

As illustrated by table 1.1, 1.2 and 1.3, a large diversity of aquatic animals produce sounds in various contexts. Yet, a lot remains unknown in the world of freshwater sound production. Several directions can be explored to extend the knowledge. In an acoustic monitoring perspective, the main goal would be to associate sounds to species, enabling to identify species according to their sound production. To plan an efficient and quick gain in knowledge concerning the sound production, it is necessary to prioritize some groups. In this section, we list the taxonomic groups which are known to produce sounds but are lacking a formal description, and then we state the additional groups which are most likely to produce sounds in freshwater environments.

1.4.1 Sound emitters lacking a formal description

In amphibians, all the reproductive calls are rather well described. However, the other less conspicuous call types such as alarm or release calls are usually known to exist but not always properly described.

In crustaceans, the sounds produced by the Louisiana Crayfish have partly been described. However, this species is awaiting a description of the frequency (or pseudo frequency) components.

In 29 genera of insects (out of 36), signals have been reported but never described in terms of their frequency range and time characteristics (Table 1.3). According to the number of species in those genera, 180 species await a formal description of their sound production.

1.4.2 Other promising sources ?

The general tendency not to report unsuccessful experiments in scientific publication (Scargle, 1999) usually renders the extend of exploration that a "non sonic" species has benefited from unknown. Species produce sounds at specific times, places and frequencies (Jansson, 1974; Cott et al., 2014). Extensive and continuous recordings with highly sensitive hydrophones are thus needed to explore more thoroughly such places or times or to capture very low amplitude sounds that might have been missed in non continuous surveys. For example, most Corixidae produce sound during the night, therefore trying to record them during the day is likely to be unsuccessful (Jansson, 1974). Moreover, one sound produced during copulation in *Sigara dorsalis* is much fainter than the other calls, and has proven to be difficult to record except in an aquarium (Jansson, 1979a).

As most amphibians produce air-borne sounds, few studies have looked at the underwater components of their calls. Indeed a lot of species produce sounds at the water/air interface and some mostly underwater (Duguet and Melki, 2003), therefore extending the use of hydrophones to record amphibians might uncover new acoustic displays.

Although sound production in Crustaceans is not well studied (Favaro et al., 2011), it has already been described and characterized in a few marine species: snapping shrimps (Kim et al., 2010), lobsters (Bouwma and Herrnkind, 2009), and crabs (Boon et al., 2009). It could be worth investigating closely related freshwater species such as freshwater crabs in the families Grapsidae or Potamonidae. In freshwater, two species of crayfish worldwide are known to produce sounds (Sandeman and Wilkens, 1982; Favaro et al., 2011), and more crayfishes could be expected to produce sounds.

Fish acoustic communication has been mainly studied in marine species (*e.g.* toadfish, Maruska and Mensinger 2009; clownfishes, Colleye and Parmentier 2012), however, there is also some data on freshwater fish (Table 1.2). The work on freshwater fish is relatively recent and has mostly addressed reproduction and agonistic contexts (Table 1.2). But several fishes produce sounds when handled for example (Webb et al., 2008) and there are probably sounds produced in other contexts which have not been discovered yet. Moreover, Lugli et al. (2003) found that all the Gobiidae species he studied produce sound, it is therefore likely that the investigation of the seven remaining French Gobies will unravel new sounds. In similar freshwater habitats found in North America, Anderson et al. (2008) found 62 types of sounds in the Hudson river, out of which four were precisely associated to two emitting species. They classified another 21 sounds as biological, five as non-biological and 32 as unknown. If we generalise from this example, considering that sounds classified as biological are fish sounds, only 16 % of the fish acoustic diversity in rivers might have been discovered in French rivers.

Considering the little attention that aquatic insects have benefited from, we can expect other insect families to produce sound. Especially, in Coleoptera, a group in which a wide diversity of species produce sounds but the investigation seems to remain relatively scattered (Table 1.3). 18 genera of Coleoptera include sound producing species (Table 1.3). In the families including sound producing Coleoptera, a total of 62 genera are known to occur in France. Therefore, up to 44 (62 - 18) genera, closely related to sound producing species, might be interesting to investigate. Moreover in a lot of the species investigated several sound types have been described, and up to four sound types can be distinguished in a species of aquatic bug (*Palmacorixa nana*, Aiken, 1982b). Therefore it is likely to find more than one sound type in the repertoire of aquatic insects which have already been described for sound production.

Finally, I believe that many freshwater arthropods are worth investigating, in particular those that are related to groups of terrestrial organisms demonstrated to produce sounds. For example, acoustic communication has been reported in 25 genera of terrestrial spiders (Jocqué, 2005) but no sound production has ever been reported in aquatic or semi-aquatic spiders. In French freshwater environments, two genera of spiders (*Dolomedes* and *Pisaura*) live on pond water surface and dive occasionally, and one species is fully aquatic, *Argyroneta aquatica*. Several terrestrial species in closely related families of these aquatic and semi-aquatic spiders are known for stridulation and percussion such as Hahniidae, Agelenidae and Dictynidae for *Argyroneta aquatica* and Ctenidae or Lycosidae for *Dolomedes* and *Pisaura* (Uetz and Stratton, 1982). Recording the diving bell spider (*Argyroneta aquatica*) or *Dolomedes* and *Pisaura*, may reveal some acoustic display.

1.5 Future directions

The knowledge of the mechanisms, behavioural context and timing of sound production remains highly scattered. In a context of acoustic monitoring, broadening this knowledge could enable to get information about physiological and behavioural states of the species surveyed. Moreover, knowing more about the timing of sound production is essential to plan an efficient monitoring, or to allow detecting timing anomalies such as an early start of breeding season due to a warm spring, this in turn can give hints on the potential effects of global warming (Krause and Farina, 2016). Finally, information on the morphology and anatomy of species might give insights to find new sound producing species.

Indeed, the description of a stridulatory organ by taxonomists has several times preceded the description of sound production in insects (Aiken, 1985). Therefore, studying the morphological structures could lead to unravel new sound producers in freshwater environments.

The general behavioural context of sound production is usually made explicit or described implicitly along side with the description of sound production. It is usually part of the description of the methods used to record the individuals. For example, in the first description of sound production in an aquatic insect, Frisch (1720) describes the sound produced by *Ilyocoris cimicoides* when he picked it up and brought it close to his ear. This implicitly indicates that this sound production is emitted in a stress context. This type of information is valuable in the context of monitoring to retrieve all the information contained in animal sound productions. In freshwater environments, 155 species of insects are awaiting a description of the behavioural context of their calls (Table 1.3).

Temporality of sound production is poorly known although it is highly important to know what species can be heard at what time of day, and during which season. Anurans are expected to produce sounds during reproduction, this period varies between species but is mostly during spring in temperate environments (Duguet and Melki, 2003). Moreover they are mostly active during the night (Duguet and Melki, 2003). The knowledge in fish is not clear but many species of fish reproduce during winter. Most insects are the most active and reproduce during spring and summer (Jansson, 1974; Gascón et al., 2009). Some species have been found to be active during winter under-ice (Poisson, 1957; Jansson, 1974) and most described species produce sounds during the night (Jansson, 1974). However for example for most Coleoptera which represent 182 species, no information on the seasonality or timing of calling is available.

1.6 Conclusion

Freshwater environments far from being silent environments seem to host a large sound diversity that may constitute rich and complex acoustic communities that remain unexplored. I estimate that 271 species distributed in 51 genera are known to produce sounds in French freshwater environments and could take part in acoustic communities. However, the knowledge remains incomplete: 65 % of them are lacking a formal description of the sound and 68 % are lacking a description of either their behavioural context or sound production mechanism. There is therefore still a long way before being able to associate one sound with its emitter.

Out of the 271 described species, 89% are insects. Therefore freshwater acoustic communities will be likely dominated by insects and I think that investigating insect sound production is a priority. Some sound producing species are of interest. For example, among the three orders of insects known for sound production, both Coleoptera and Trichoptera are used as ecological indicators in freshwater environments (Angélibert et al., 2010). This diversity of organisms producing sounds also includes threatened (spadefoot toad, *Pelobates fuscus*) or invasive (*Procambarus clarkii*) species.

I think that acoustic sampling is highly likely to yield valuable information on the functioning and health of the freshwater ecosystems. Two approaches to monitor habitats could be undertaken: the species specific approach and the global monitoring approach.

The species specific approach targets one species and uses sampling data, *e.g.* recordings, to evaluate the state of the population of interest. Depending on the level of knowledge, this approach can provide presence/absence data, inform on the current behavioural state of the individuals or enable to monitor dynamics of populations.

Detecting the presence of a species thanks to its sound production, requires knowledge of a species specific sound of the species of interest. The current knowledge already available for some species is sufficient to enable the detection of the presence of several species of insects, frogs and fish. However, the low level of knowledge on the timing and seasonality of calls can limit the information yielded by the lack of sound production. Moreover, the lack of formal description of many described and recorded signals highlights the importance of sound repositories. For example, the huge dataset on worldwide sounds of Corixidae and Micronectidae collected over the years by Dr. Jansson is impossible to retrieve. A sound repository for freshwater species would therefore be very helpful to mediate species identification with sound. A sound collection has recently been set up at the Muséum national d'Histoire naturelle in Paris: la sonothèque. It would be a good opportunity to use this structure to centralise freshwater data available so far.

Several sounds produced by freshwater organisms are already associated with a behavioural context, therefore identifying these sounds in freshwater recordings not only informs on the presence of a species but also on the behavioural status of this species. For example, recording the advertisement call of *Pelobates fuscus* would indicate that the individuals at this location are ready to reproduce, and recording its alarm call would indicate the presence of an imminent threat.

A systematic analysis at different time of year coupling acoustic recording and traditional sampling methods would be necessary to operationalise the use of acoustics to detect density of species. In fact, deriving density of a population from acoustic recordings necessitates to have information on the call rate of individuals in different environmental conditions. This type of estimation works best with sounds produced regularly such as contact calls. In contrast, sound productions triggered by a disturbance, such as as alarm or agonistic sounds, are likely to fail as they may be produced unevenly. This method would enable a larger spatial coverage and a continuous monitoring of populations over their period of activity.

Another approach of monitoring of freshwater habitats is the global monitoring approach. It has been developed in soundscape ecology and eco-acoustics around the idea that the sound diversity, structure and composition found in an environment can be informative on the type of habitat in which the sounds were recorded (Fuller et al., 2015; Gasc et al., 2013). It has thus been shown that acoustic diversity is associated with biological diversity in several previous studies undertaken in tropical and temperate forests (Sueur et al., 2008; Pieretti et al., 2011). However further studies have shown that the relationship between the acoustic and taxonomic diversity can be more complex, especially because of noise due to natural abiotic perturbation which increase the background noise, such as wind or rain (Depraetere et al., 2012). We wonder whether acoustic diversity could also be related to biological diversity in freshwater environments. This is an idea which we will develop in the next chapter.

1.7 Acknowledgments

We thank Romain Garrouste for sharing with us interesting field sites. We also thank the Office National de Forêts du Ruscas, the Station Biologique de Foljuif, the Fondation d'entreprise du golf de Vidauban, Joëlle and Gerard Lefrançois for letting me record and collect insects on their properties.

1.8 Personal recordings

1.8.1 Objectives

We decided to record isolated species of aquatic insects and crustacean in the laboratory to expand our knowledge.

1.8.2 Methods

The recorded individuals were collected in different ponds in the surroundings of Paris, in the south of France (Var (83)) and in Normandie (Seine-Maritime (76)).

The recording set up consisted of three units: (1) a hydrophone Reson TC4033 (flat frequency response between 20 Hz and 40 kHz) with a 10 meter cable, (2) a charge pre-amplifier Avisoft UltraSoundGate with a frequency high-pass filter at 100 Hz and a gain of +20 dB, (3) a portable recorder Tascam DR-100. The recording set up was installed in an aquarium containing one or several individuals of the same species. When possible, the individuals were sexed after recording and death, stored in alcohol and identified to species level.

The recorder was set up to record continuously during a period of duration varying from 1 hour to more than four days. The audio files were stored on SD memory cards as uncompressed .wav files at a 44.1 kHz sampling frequency and a 16 bit digitization depth.

Aural inspection was achieved using circumaural headphones and by listening to the files as many times as necessary. This aural inspection was accompanied with the sound visualisation of oscillograms and spectrograms (Window length: 512 samples, frame overlap: 50%, window type: Hanning) with the software Audacity (Mazzoni, D., http://audacity.sourceforge.net/). The detected sounds were annotated, extracted and associated to the emitting species.

1.8.3 Results

We recorded a total of 19 different species including five species of crustaceans and 14 species of insects on a total duration of 487 hours (Table 1.4) including five species of crustaceans and 14 species of insects. Nine of these 19 species were previously reported to produce sounds. In the laboratory, eight species were found to produce sounds and among them, six were already reported in the literature to produce sounds and two had never been observed to produce sounds before. Therefore three of the soniferous species did not produce sounds in the conditions of our laboratory.

The total percentage of the files containing sounds was 7 %. In files with sounds, this percentage reached 18 %. We can note the high percentage of sound production by *Micronecta* species reaching 75% for *Micronecta griseola* and 79% for *Micronecta scholtzi*.

Table 1.4 – **Results of recordings in the laboratory.** In the column *Sex and number of individuals*, F corresponds to the number of females and M to the number of males. F/M means that females and males were not identified, n means that there was between ten and twenty individuals.

Species	**Known for sound production**	**Sex and number of individuals**	**Recording duration (days)**	**Total duration of detected sounds (s)**	**Percentage of the recorded file containing sounds**
Acilius sulcatus	yes	F:0, M:1	0.111	308.606	3.212
Acilius sulcatus	yes	F:0, M:1	0.666	0	0
Acilius sulcatus	yes	F:1, M:0	0.388	0	0
Atyidae	no	F/M:n	0.521	0	0
Austropotamobius pallipes	no	F:0, M:1	0.078	213.282	3.172
Austropotamobius pallipes	no	F:0, M:1	0.061	0	0
Berosus sp.	yes	F/M : 2	0.039	0	0
Chirocephalus sp.	no	F/M:n	0.14	0	0
Chirocephalus sp.	no	F/M:n	0.11	0	0
Colymbetes fuscus	no	F:1, M:0	0.741	0	0
Colymbetes fuscus	no	F:0, M:1	0.702	0	0
Colymbetes fuscus	no	F:1, M:0	0.749	0	0
Copelatus haemorhoidalis	no	F/M:1	0.755	0	0
Corixa punctata	yes	F:3, M:3	0.26	0	0
Corixa punctata	yes	F:3, M:3	4.559	0	0
Gammarus sp.	no	F/M:n	0.743	2286.125	3.559
Gammarus sp.	no	F/M:n	0.686	0	0
Hespecorixa sahlbergi	yes	F:3, M:0	0.731	31.203	0.049
Hespecorixa sahlbergi	yes	F:2, M:0	1.005	282.958	0.326
Hespecorixa sahlbergi	yes	F:0, M:1	0.706	94.146	0.154
Hespecorixa sahlbergi	yes	F:0, M:5	0.739	36.915	0.058
Hydaticus seminiger	no	F/M:1	0.769	0	0
Hydrophilus piceus	no	F/M:1	0.703	0	0
Micronecta griseola	yes	F/M:n	0.695	45093.412	75.072
Micronecta scholtzi	yes	F/M:n	0.957	65232.969	78.9
Naucorus maculatus	yes	F/M:5	0.118	0	0
Notonecta sp.	no	F/M:3	0.699	0	0
Plea minutissima	yes	F/M:n	0.777	1098.3	1.637
Sigara striata	yes	F:1, M:2	0.738	993.074	1.557
Triops cancriformis	no	F/M:4	0.351	0	0

For each species, a spectrogram and an oscillogram of the sounds recorded are presented below.

1.8.3.1 Crustaceans

Two crustacean species were recorded: Figure 1.10 and 1.11.

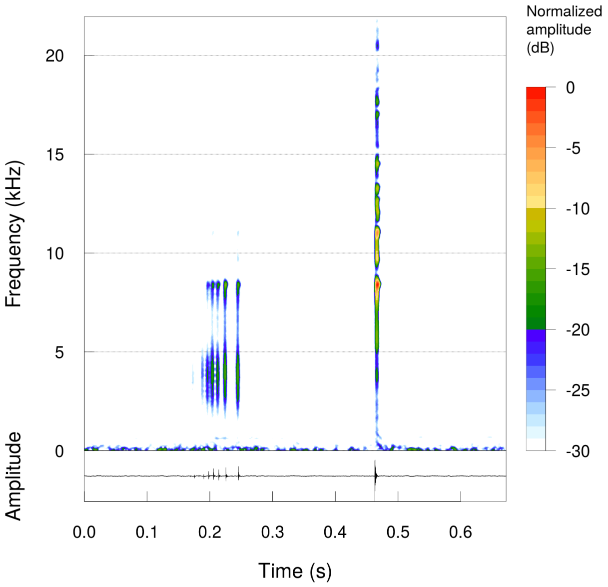

Figure 1.10 – **Sound production by the white-clawed crayfish (*Austropotamobius pallipes*)**. This sound was emitted when the individual was disturbed by a human observer with a small stick.

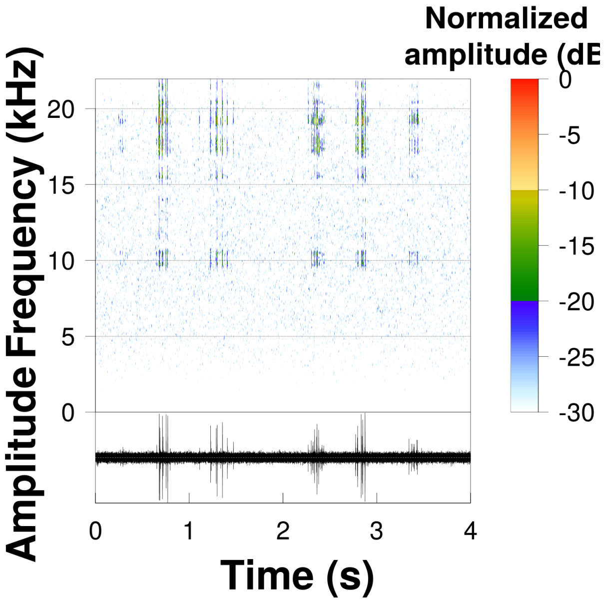

Figure 1.11 – **Sound production by *Gammarus* sp.**

1.8.3.2 Insects

Six insect species were recorded: Figure 1.12-1.17.

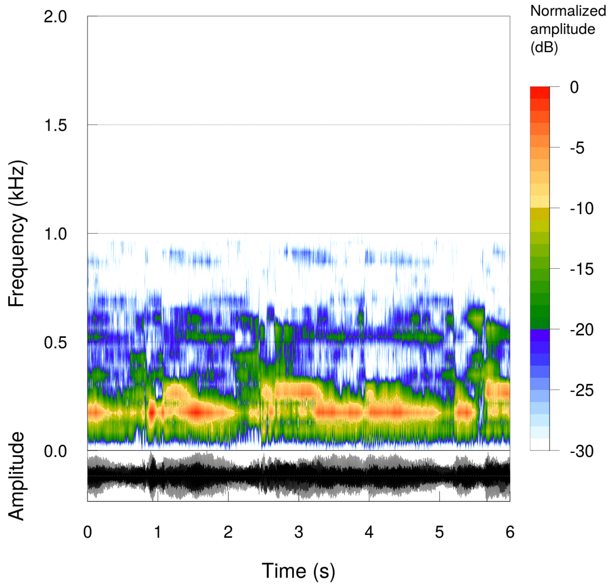

Figure 1.12 – **Sound production by a Dytiscid water beetle (*Acilius sulcatus*).** This low frequency sound was recorded before the individual tried to fly out of his aquarium, and according to Leston et al. (1965), this sound corresponds to flight muscle contraction in preparation for flight.

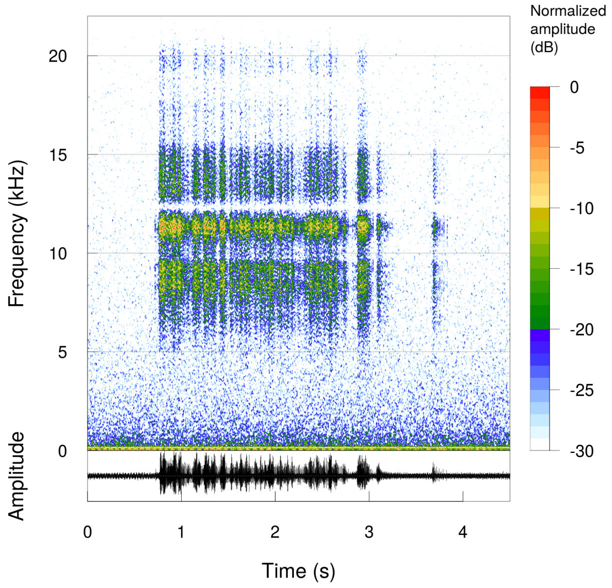

Figure 1.13 – **Sound production by the pygmy backswimmer (*Plea minutissima*).** This sound was described in Wefelscheid (1912), as a "slight rubbing over a fine file", and is supposed to be emitted by the rubbing of striated areas located on the underside of the abdomen of both males and females.

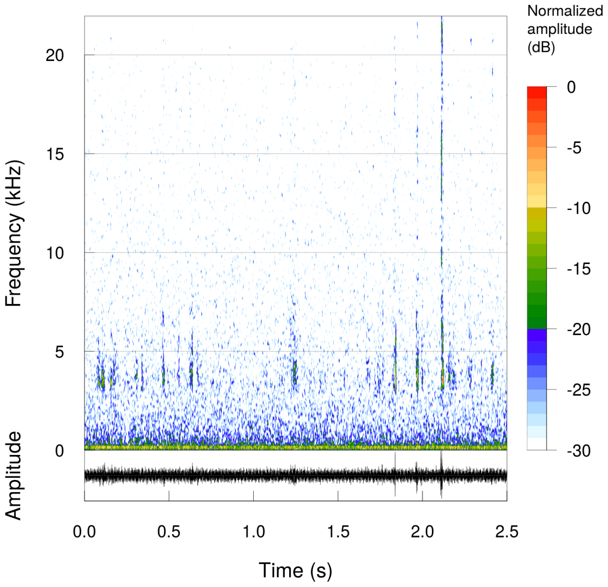

Figure 1.14 – **Sound production by a water boatman (*Hespecorixa sahlbergi*).** A similar sound was decribed in other species of *Hespecorixa* (Aiken, 1985).

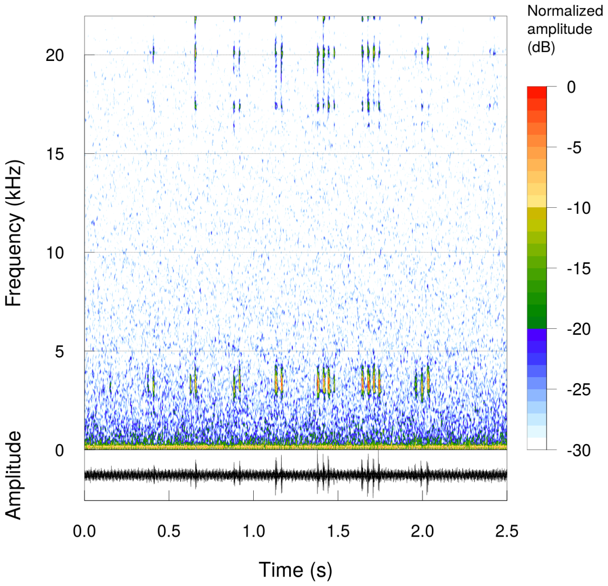

Figure 1.15 – **Sound production by a water boatman (*Sigara striata*).**

1.8.4 Conclusion and perspectives

We found that 7 % of the recorded time contained sound productions. Some species such as the species of *Micronecta* produce sound almost continuously while other species emit very few sounds. This low level of soniferous activity for species that have already been reported to produce sounds could be linked to the stress induced by laboratory conditions.

The spectrograms and oscillograms revealed low levels of SNR, especially for *Plea minutissima*, *Gammarus* sp. or *Sigara striata*. In these species, sound production appears

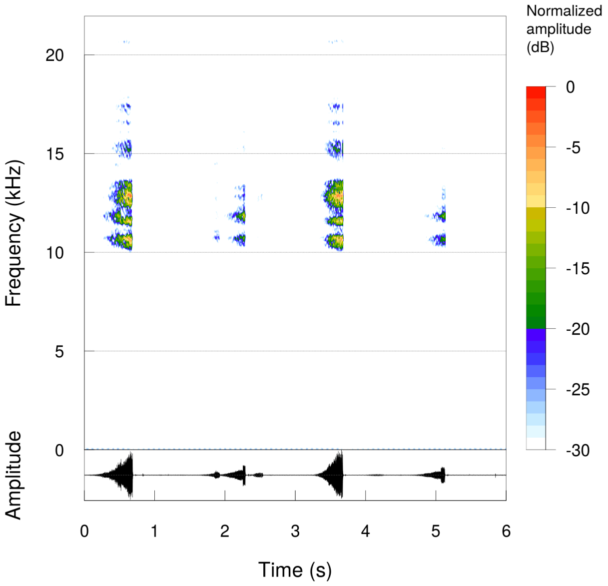

Figure 1.16 – **Sound production by a pygmy water boatman (*Micronecta griseola*)**. This sound was reported in Jansson (1989). Hypothesis on this sound includes sexual and aggregative functions.

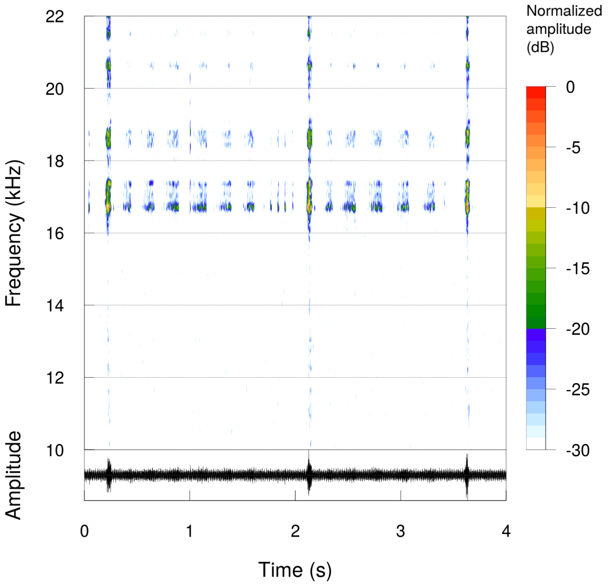

Figure 1.17 – **Sound production by a pygmy water boatman (*Micronecta scholtzi*)**. This sound was reported in Sueur et al. (2011). Hypothesis on this sound includes sexual and aggregative functions.

relatively faint.

Moreover, some frequency appear as trails on the spectrograms (Figure 1.17). These trails correspond to the resonant frequencies of the aquarium (Akamatsu et al., 2002). The resonant frequencies are problematic to assess the dominant frequencies of the recorded species in aquariums. To precisely assess frequency features it might be important to record the individuals in their natural habitats as well as in aquariums.

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Chapter 2 :

First description of underwater acoustic diversity in three temperate ponds

Hydrophone set up in pond 2, Vallée de Cheuvreuse (Yvelines, 78), June 2010
(Picture: Amandine Gasc)

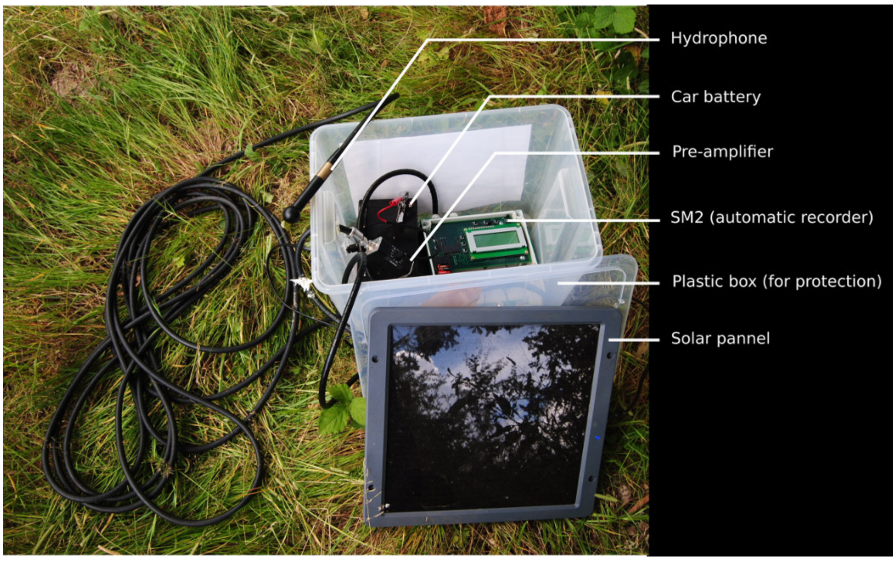
2.1 Summary

Context Although several species are soniferous in freshwater environments, the way the diversity of soniferous species is structured into acoustic communities has never been explored in ponds.

Problematics

- How does the acoustic diversity differ in richness and composition between three temperate ponds in different habitats ?
- Does the richness of acoustic diversity vary along the day revealing cycles of presence and activity in the soniferous species ?
- Can acoustic indices be calculated and used to measure and reflect the richness of acoustic diversity of each pond ?

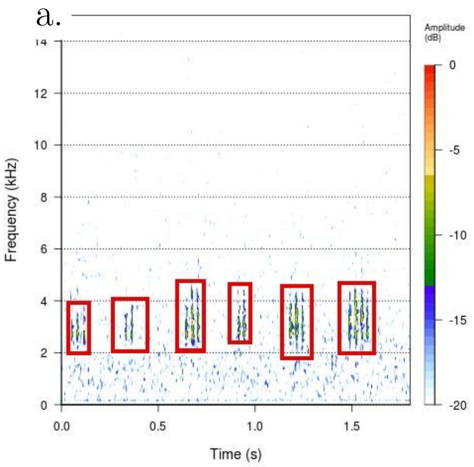
Methods Long-term underwater acoustic monitoring in three temperate ponds located in three different habitats:



- Recording from June to September 2010
- Recording schedule: 1 minute every 4 hours
- 360 analysed files

Figure 2.1 – **Picture of the automatic recording device showing the different components of the recorder**. This includes an automatic recorder, a pre-amplifier, a hydrophone and a solar panel. The equipment is enclosed in a plastic box avoiding any damage to electronic devices due to precipitations.

Figure 2.2 – **Three study ponds** (pictures: Marion Depraetere). a. Closed forest habitat (pond 1), b. Open field habitat (pond 2), c. Semi-closed habitat (pond 3).



b.

$$H(X) = -\int_{\infty}^{\infty} p_X(x) \times \log_2[p_X(x)]\mathrm{d}x$$

$$ACI_t = \frac{\sum_{k=1}^{n-1} \mid I_k - I_{k+1} \mid}{\sum_{k=1}^{n} I_k}$$

- Extraction of sound type composition and richness per recording
- Application of six acoustic diversity indices to the recordings
- Correlation tests between acoustic indices and sound type richness
- Correspondence analysis to study acoustic composition
- GLMM to investigate the effects of time on the richness of acoustic diversity

Figure 2.3 – **Two methods to analyse the recordings**. a. Visual identification of sound types. b. Estimate of the acoustic diversity by acoustic indices calculation.

Main results

- 48 different sound types recorded and identified in the three ponds
- The three ponds located in three different habitats host distinct acoustic communities

- Daily variation of the acoustic diversity in the three ponds
- Among the six acoustic indices tested, only one, the Acoustic Richness index (AR) was not correlated with SNR revealing that SNR could be an important confounding variable for the five other acoustic indices

Perspectives A species-specific approach based on behavioural and systematic sciences to identify the sound sources should be undertaken along with a community approach based on ecological sciences to allow rapid biodiversity assessment.

Related communications and publications

- First description of underwater acoustic diversity in three temperate ponds. **Camille Desjonquères**, Fanny Rybak, Marion Depraetere, Amandine Gasc, Isabelle Le Viol, Sandrine Pavoine, Jérôme Sueur. 2015. *Peer J*, 3:e1393

2.2 Introduction

Over the past 15 years, scientists and land managers have started to draw the attention on the importance of ponds in terms of patrimonial, recreational, decorative, agricultural, ecological and environmental interests (Oertli et al., 2009). These water bodies are occupied by a large diversity of organisms harbouring an important number of endemic vertebrate and invertebrate species, some of which are highly threatened (Céréghino et al., 2012). Natural processes such as sedimentation or seasonal changes modify ponds and their environmental parameters, they are thus most of the time bound to be temporary (Wood et al., 2003). Ponds are experiencing an unprecedented and severe degradation due to anthropological causes such as drainage, ancient custom abandon (*e.g.* forest or agricultural ponds), urbanisation or agriculture intensification (Wood et al., 2003). This degradation can take several forms such as habitat fragmentation, reduction, quality alteration (*e.g.* pollution or desiccation) or even complete habitat loss. Hence, depending on the area, 40 to 90% of European ponds have disappeared during the twentieth century (United Kingdom: Wood et al. (2003), Europe: Hull (1997)). Combining a unique diversity and a high level of threat, many ponds can be therefore considered as habitats of high concern for biodiversity conservation, in particular under temperate climates (Verberk et al., 2006).

Plant and animal diversity of ponds has been well studied, however an original facet of biodiversity, the acoustic diversity due to the acoustic signals produced by animals, has been totally neglected. The description and analysis of acoustic diversity was proven to be a valuable approach of diversity assessment combining results in animal behaviour and ecology, in particular to estimate the space and time distribution of species (Towsey et al., 2014a). The acoustics of freshwater ecosystems have rarely been investigated. Yet they are inhabited by many species belonging to taxa for which terrestrial as well as some aquatic members are known to produce sounds. In ponds, a high diversity of amphibians generates sound underwater during breeding (Duguet and Melki, 2003). Sound production in freshwater arthropods is also quite common but has not been given as much attention. A few crayfish species have been proven to produce sound (Sandeman and Wilkens, 1982; Favaro et al., 2011), most acoustic diversity in ponds is probably due to insect stridulation (Aiken, 1985). A comprehensive review of sound-producing aquatic insects lists 15 families belonging to four orders, namely Trichoptera, Odonata, Coleoptera and the order with the largest number of sound-producing species, Heteroptera (Aiken, 1985). The underwater insect signals cover a wide frequency bandwidth, ranging from 200 Hz for the Coleoptera imago Acilius sulcatus (Leston et al., 1965) up to 100 kHz for Hydropsychidae larvae (Trichoptera, Silver and Halls (1980)).

Sounds produced by animals can be considered as interacting items belonging to a high-level ecological organization such as a community or a landscape (Farina, 2014). Ecoacoustics, a newly formed discipline, aims at considering animal sound as a material for ecology and biodiversity monitoring (Sueur and Farina, 2015). In this global approach, sound is mainly considered as a tool to infer ecological information. In practice, recordings do not focus on a single singing species but on the overall acoustic output emanating from a community or a landscape. The analysis of these sounds aims to assess and characterize general features of the structure and the diversity of either an acoustic community, defined as an assemblage of species that share a similar acoustic space (Gasc et al., 2013) or a soundscape, defined as 'the collection of biological, geophysical and anthropogenic sounds that emanate from a landscape' (Pijanowski et al., 2011). The global approach is mainly based on the assumption that part of biodiversity can be reflected by acoustic diversity. Numerous acoustic diversity indices have been proposed to measure the acoustic community diversity or soundscape composition (Sueur et al., 2014). All these indices forego species identification and produce relative values that aim at quantifying a feature of the community or the soundscape, like the energy, the complexity or the relative importance of the biophony. The indices have been first tested in terrestrial environments (Sueur et al., 2008b; Pieretti et al., 2011; Gage and Axel, 2014; Towsey et al., 2014b) and then in marine habitats (Parks et al., 2014). These first trials revealed mixed results suggesting the importance of background noise in the reliability of indices (Gasc et al., 2015).

No passive acoustic monitoring study considering all sources of acoustic productions had been conducted in freshwater ponds. We thus explored for the first time the acoustic diversity of three temperate ponds in three different habitats. We tested the following hypothesis: (i) the acoustic diversity differs in richness and composition between the three ponds, (ii) the richness of acoustic diversity varies along day and night revealing ecological cycles, and (iii) acoustic indices can represent the richness of acoustic diversity of each pond detected by human-hearing.

2.3 Materials and methods

2.3.1 Study area and recordings

Three ponds were monitored in the Parc Naturel Régional de la Haute Vallée de Chevreuse (PNR), a protected area located 40 km south-west of Paris, France. The ponds were located in three different environments differing by the density of the surrounding vegetation: closed forest habitat (pond 1, 48°34.523'N, 1°53.341'E), semi-closed habitat (pond 3, 48°40.772'N, 1°55.840'E) and open field habitat (pond 2, 48°40.560'N, 1°55.865'E). The main characteristics of the three ponds are summarised in Table 6.1.

Each pond was monitored with an autonomous recording platform including four units:

(1) a hydrophone Reson TC4033 (flat frequency response between 20 Hz and 40 kHz) with a 10 meter cable, (2) a charge pre-amplifier Avisoft UltraSoundGate with a frequency high-pass filter at 100 Hz and a gain of +20 dB, (3) a digital audio field recorder SM1 (Wildlife Acoustics, 2009) with a built-in Texas-Instrument anti-alias filter, (4) a 12 V battery connected to the audio recorder and charged with a solar panel. A four-point linear transect was defined to cross each pond to maximize representation of heterogeneous patches of vegetation. Recordings were achieved on each of the four points of this transect to collect spatial heterogeneity within the ponds. A single recording platform was available for each pond. The hydrophone position was changed every three days according to a sampling rotation with a set up allowing the hydrophone to move without any intrusion in the ponds. The hydrophone was placed 10 cm below the water surface to reduce heterogeneity of sound propagation that is depth-dependent in shallow water (Forrest et al., 1993). Rainfall data were collected from a local meteorological station (Météo France, 2010).

Each recorder was programmed to record during one minute every fifteen minutes between the 23rd of June and the 15th of September 2010 (84 days) when the activity and abundance of macro-invertebrate species are known to be maximal (Gascón et al., 2009). The sampling design (3 ponds x 96 recording time slots x 84 days) resulted in $24,192$ files, among which $7,873$ were missing due to technical issues (file corruption, material theft and dysfunction). The final number of files obtained was $16,319$. These recordings were sampled at 44.1 kHz with a 16 bits digitization. The files were saved in the lossless compressed format .wac and then transformed into the format .wav with the software WAC to WAV Converter Utility version 1.1 (Wildlife Acoustics, 2009).

2.3.2 Aural and visual classification of sounds: detection of sound types

Visual identification was conducted on a sub-sample of the initial sample of 16 319 files. Five complete recording days for each sampling point in the three ponds were randomly selected avoiding rainy days. For each selected day, six recording times were defined (00:00, 04:00, 08:00, 12:00, 16:00 and 20:00) resulting in 360 recordings (3 ponds x 4 sampling points x 5 days x 6 recordings per day). These 360 recordings can be considered as samples. 28 recordings, spread across the 3 ponds, had to be withdrawn from this sub-sample due to technical problems with the recorders. Due to the lack of a sound bank for most freshwater species, sounds could not be identified at a species level. Therefore sound types, instead of species-specific sounds, were identified and classified based on aural and visual inspections. Aural inspection was achieved using circumaural headphones and by listening to the files as many times as necessary. This aural inspection was accompanied with the sound visualisation of oscillograms and spectrograms (Window length: 256 samples, frame overlap: 0%, window type: Hanning) with the software Audacity (Mazzoni, D., http://audacity.sourceforge.net/). The classification of sound types was based on similarity in amplitude and dominant frequency contours and achieved only by MD to avoid any bias due to the experimenter. This identification of sound types was summarized by two variables for each recordings: i) the richness of sound types per recording (hereafter referred to as richness) which is the number of different sound types in a recording and ii) the abundance of sound types per recording (hereafter referred to as abundance) which is the total number of sound types detected in a recording.

2.3.3 Signal to noise ratio (SNR)

The signal-to-noise ratio (SNR) of each recording was estimated by computing the ratio between the amplitude of one second of signal (extract lasting one second and containing an identified sound type) and the amplitude of one second of noise (e. g. one second of recording without any signal) in each file as follows:

$$SNR = \frac{{A_s}^2}{A_n} \tag{2.1}$$

with A_s and A_n the root mean square (RMS) of signal and noise sections respectively. dB values were obtained by computing:

$$SNR_{dB} = 20 \times log_{10}(SNR) \tag{2.2}$$

The SNR of recordings in which no signal could be found was set to one leading to a SNR_{dB} of zero.

2.3.4 Acoustic analysis

Several acoustic indices have been developed recently to assess the acoustic diversity of a community or a landscape (Sueur et al., 2014). Six acoustic indices were chosen to parametrize the files that were aurally and visually inspected. These indices were (1) the temporal entropy H_t that computes the Shannon evenness of the amplitude envelope (Sueur et al., 2008b), (2) the spectral entropy H_f that computes the Shannon evenness of the mean frequency spectrum (Sueur et al., 2008b), (3) the envelope energy M that returns the median of the amplitude envelope (Depraetere et al., 2012), (4) the acoustic richness AR which is a ranked index based on the multiplication of H_t and M (Depraetere et al., 2012), (5) the number of major peaks of the mean frequency spectrum NP with an amplitude threshold of the slopes of 1/50 and a frequency threshold of 200 Hz (Gasc et al., 2013), and (6) the Acoustic Complexity Index ACI which calculates the complexity of the spectrogram, i.e. of the short-term Fourier transform (Pieretti et al., 2011). More details regarding these indices can be found in Sueur et al. (2014). All spectral data were obtained with a short-term Fourier transform with a 512 samples non-overlapping Hamming window. To obtain H_t, the absolute amplitude envelope was computed. For NP the parameters used were 1/50 for the amplitude slope threshold and 200 Hz for the frequency threshold. The time step for the ACI was 30 seconds and the frequency bins were 2 Hz. All acoustic analyses were achieved with the package seewave (Sueur et al., 2008a) of the R statistical environment (R Core Team, 2015).

2.3.5 Statistical analysis

Richness in sound types were compared between ponds with sample-based rarefaction curves (Simberloff, 1972) obtained with the R package vegan version 2.2-1 (Oksanen et al., 2013).

A Correspondence Analysis (CA) was computed to characterize the community at each recording point along the transect and the way the communities were ordinated considering their sound type composition. Each recording point was considered as a single observation and the presence/absence data for each sound type was used as a variable.

Differences in SNR between ponds were first assessed with an analysis of variance (ANOVA) but the assumptions of normality and homoscedasticity of the model residuals were not met. A Kruskal-Wallis non-parametric test was therefore used followed by pairwise comparisons using Wilcoxon rank sum test with a Bonferroni adjustment.

To investigate the effect of time in the ponds on sound type richness per recording, we used a Generalized Linear Mixed Model (GLMM; Baayen, 2008) with a Poisson error structure and log link function (McCullagh and Nelder, 1989). To examine the daily cyclic effects of time on the richness, we transformed time into a circular variable and included its sine and cosine into the model (Cox, 2006). Since the effect of time is likely to vary among ponds if they host different species, we included the interaction between pond and the sine and cosine in the model. Transect point and recording day were included as random effects. To keep type I error rate at the nominal level of 5% (Schielzeth and Forstmeier, 2009; Barr et al., 2013) we included all possible random slopes components (sine and cosine of time within both transect point and recording day and pond within recording day) and also the respective correlations between random slopes and intercepts. As an overall test of the fixed effects, we compared the full model with a null model lacking the fixed effects but comprising the same random effects structure as the full model (Forstmeier and Schielzeth, 2011) using a likelihood ratio test (Dobson and Barnett, 2008). We assessed model stability by comparing the estimates derived by a model based on all data with those obtained from models with the levels of random effects excluded one at a time. This revealed the model to be stable. To rule out collinearity we determined Variance Inflation Factors (VIF, Field, 2005) for a standard linear model excluding random effects and interactions. It revealed a VIF of 1.000 for sine and cosine time and pond which means that there was no collinearity issue.

As data were not normally distributed, correlations between indices and aural analysis were calculated using Spearman's formula. To investigate relationships between acoustic indices and richness and abundance of sounds aurally and visually determined, we performed correlations between acoustic indices (H_t, H_f, M, AR, ACI and NP) and the richness and abundance of sound types. To take into account the effect of the noise on the indices we first estimated the correlation between the acoustic indices (H_t, H_f, M, AR, ACI and NP) and the SNR. Then to control for the correlation between the noise and the indices when investigating the relationship between indices and sound type richness and abundance, we used partial Spearman correlations controlling SNR (Kim, 2012).

All statistical analyses were run with the R statistical environment (R Core Team, 2015) with the packages ade4 version 1.7-2 (Dray and Dufour, 2007), ppcor version 1.0 (Kim, 2012), lme4 version 1.1-7 (Bates et al., 2014) and car version 2.0-25 (Fox and Weisberg, 2011).

2.4 Results

2.4.1 Pond acoustic richness

A total of 2 446 sounds were detected and allocated to 48 sound types (see 3 examples in Figure 2.4). 42 sound types were identified in pond 1, 22 in pond 2, 9 in pond 3. Pond 1 and pond 2 shared 18 sound types, pond 1 and 3 shared 6 sound types and ponds 2 and 3 shared 7 sound types. The rarefaction curve showed a plateau for both pond 2 and 3 but not for pond 1 (Figure 2.5). The plateau is reached at 22 sound types for pond 2 and 9 for pond 3.

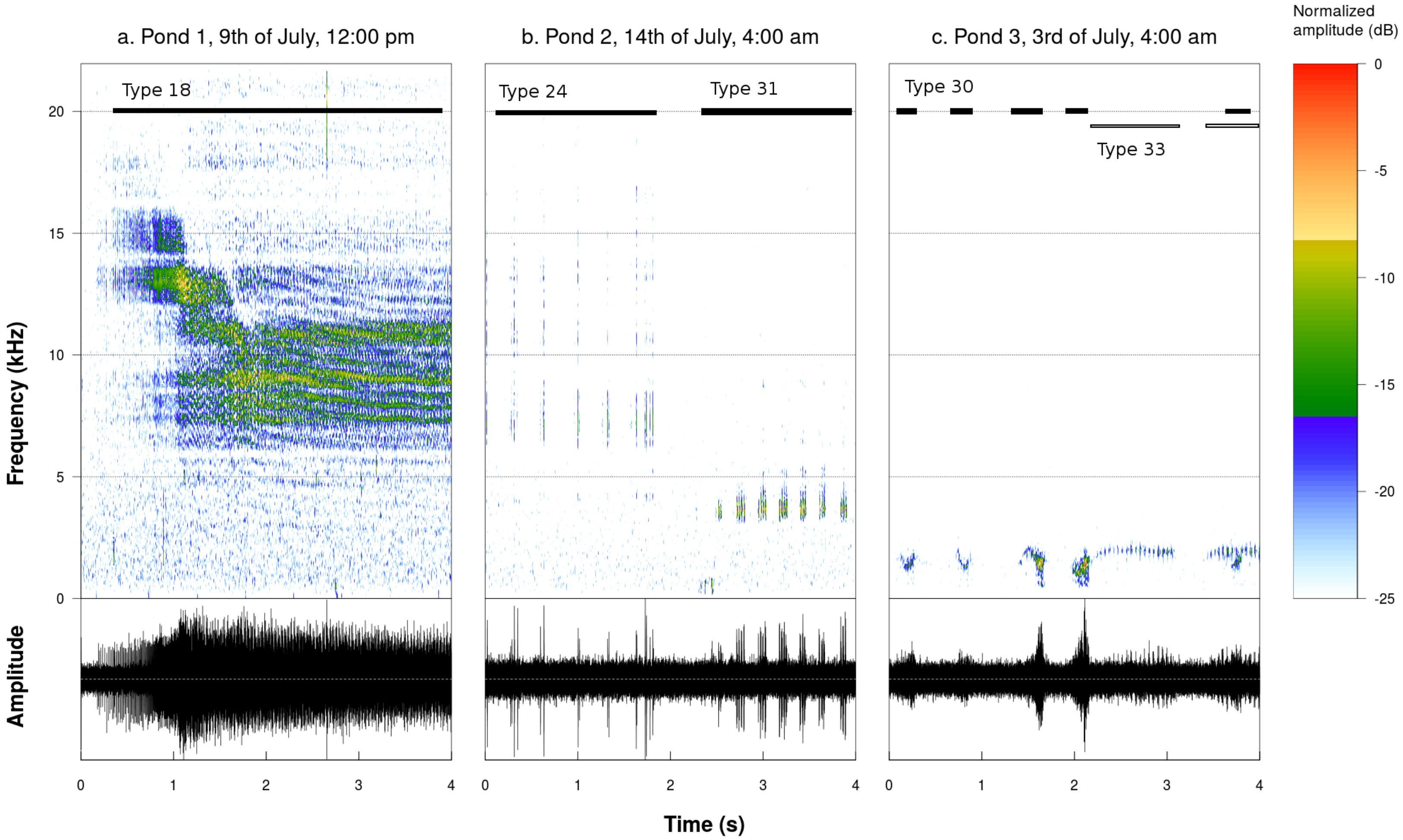


Figure 2.4 – **Spectrograms and oscillograms of chosen sound productions illustrating the acoustic diversity found in the studied ponds (Fourier window length: 1,024 samples, frame overlap: 50%, window type: Hanning).** a. Sound type 18 recorded in pond 1 on the 9th of July at 12:00 pm. b. Sound types 24 and 31 recorded in pond 2 on the 3rd of July at 4:00 am. c. Sound types 30 and 33 recorded in pond 3 on the 14th of July at 4:00 am.

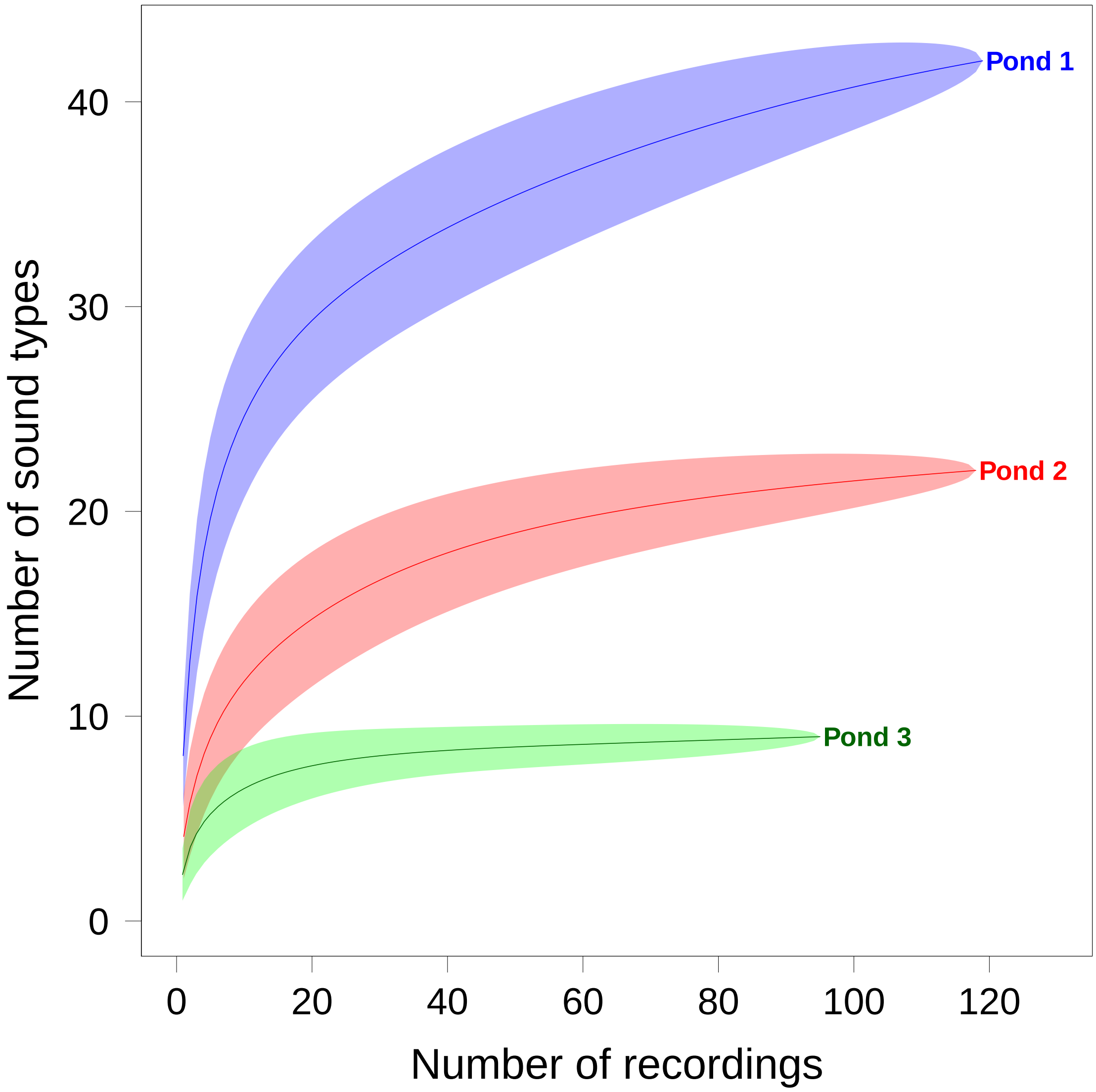


Figure 2.5 – **Sample based rarefaction curves of sound types per recording for each pond.** Each one minute recording is a sample and the sound types are equivalent to species in the rarefaction process. Shaded area around the curves indicates 95% confidence intervals.

The mean number of sound types found in a recording was 2.2 ± 1.8 (mean ± sd, $n = 119$) for pond 1, 1 ± 1.1 ($n = 118$) for pond 2 and 0.6 ± 0.8 ($n = 95$) for pond 3. The recording points were more similar within ponds than among ponds as shown by the projection of recording sites along the axes 1 and 2 of a CA (Figure 2.6). The two first axes explained 43% of the total variance.

There was an impact of pond and time on the richness (likelihood ratio test comparing the full and null model including only the factor pond, $\chi^2 = 17.269$, $df = 8$, p-value= 0.027). The daily variation was different from one pond to the other (significant interaction between ponds and time, likelihood ratio test comparing the full model and the model

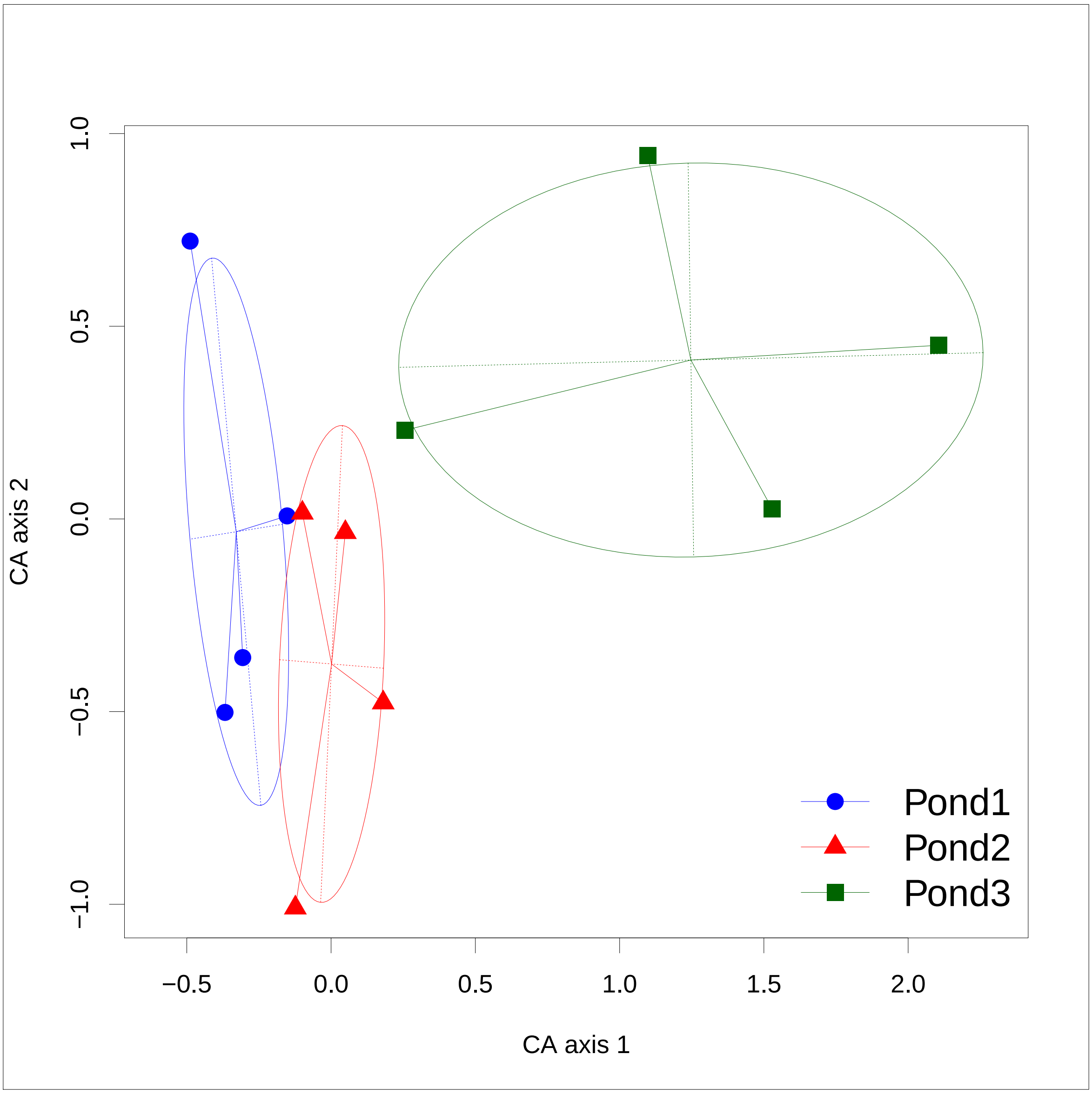


Figure 2.6 – **Result of the Correspondence Analysis (CA) with the sound types as variables and the recording points as samples.** Each point represents a point of recording in the pond, each ellipse corresponds to 67% of the point dispersion around the centroid for each pond. The axes 1 and 2 explain 26% and 17% of the variance, respectively.

Table 2.1 – **Spearman correlations between acoustic indices (H_t, H_f, M, AR, ACI and NP) and richness and abundance of sound types and SNR.** Stars indicate the significance of the correlation test. Bonferroni adjusted p-value *<0.05, **<0.01, ***<0.001.

	H_t	H_f	M	AR	NP	ACI
SNR	-0.61 ***	-0.41 ***	0.48 ***	-0.19 *	0.42 ***	0.55 ***
richness	-0.5 ***	-0.3 ***	0.44 ***	-0.04	0.34 ***	0.49 ***
abundance	-0.53 ***	-0.34 ***	0.47 ***	-0.06	0.36 ***	0.5 ***

without the interaction, $\chi^2 = 10.117$, $df = 4$, p-value= 0.039). Using the predictions of the model, we found that pond 1 had an overall higher number of sound types with its highest sound richness at 11:18, pond 2 had an intermediate sound type richness and its highest sound richness at 20:00 and finally pond 3 had the lowest sound type richness with its highest sound richness at 16:22 (Figure 2.7).

2.4.2 Level of SNR

The values of the signal-to-noise ratio had a mean of 5.38 equivalent to 7.31 dB and a median of 1.12 equivalent to 0.5 dB. These values were variable with a standard deviation of 24.51 equivalent to 14 dB, and a median absolute deviation of 1.67 equivalent to 2 dB. 137 out of 332 recordings (41%) had a SNR lower than 1 equivalent to 0 dB. The SNR levels differed significantly between pond 1 and 2 and pond 1 and 3 but not between pond 2 and 3 (pairwise Wilcoxon test, p-values adjusted with the Bonferroni correction: pond 1 – pond 2 > 0.0001, pond 1 – pond 3 > 0.0001, pond 2 – pond 3 $= 0.33$, $n = 332$). Pond 1 had a significantly higher SNR.

2.4.3 Correlation with the acoustic indices

Correlations between indices and aural analysis revealed that the indices H_t and H_f were negatively correlated with the SNR, the sound type richness and the sound type abundance (Table 2.1). The indices M, ACI and NP were positively correlated with the SNR and both sound type richness and abundance. The index AR was negatively correlated with SNR but was not correlated either with sound type richness or abundance (Table 2.1). The richness and abundance of sound types were correlated with the SNR (respectively 0.80 and 0.84, p-values < 0.001). Partial correlations given the SNR revealed that AR was positively correlated with both the richness and abundance of sound types. All other indices were not significantly correlated with either abundance or richness given the SNR (Table 2.2).

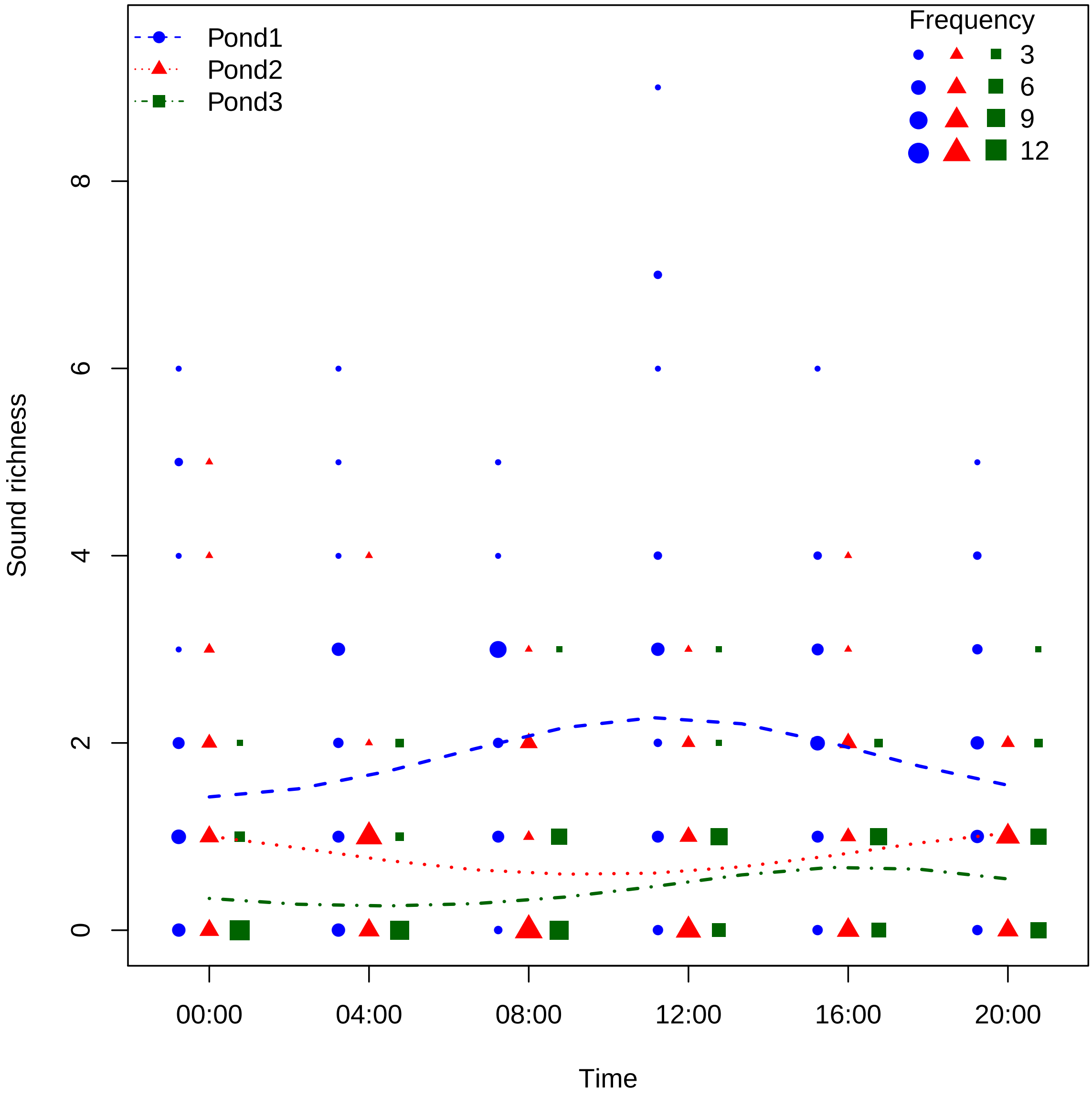


Figure 2.7 – **Frequency distribution of sound type richness as a function of time.** The size of each point is related to the number of recordings containing the same number of different sound types (total number of recordings N = 332). The three dashed lines show the fitted model for each of the three ponds.

Table 2.2 – **Spearman partial correlations between acoustic indices (H_t, H_f, M, AR, ACI and NP) and richness and abundance of sound types given the SNR.** Stars indicate the significance of the correlation test. Bonferroni adjusted p-value *<0.05, **<0.01, ***<0.001.

	H_t	H_f	M	AR	NP	ACI
richness	-0.02	0.06	0.11	0.2 **	0	0.1
abundance	-0.04	0	0.15	0.19 **	0.02	0.08

2.5 Discussion

The acoustic production of terrestrial and marine animals has been thoroughly studied for more than 60 years in bioacoustic studies (Fletcher, 2007). This research revealed an important sound diversity that is now reconsidered under the theoretical frameworks of soundscape ecology (Farina et al., 2014) and ecoacoustics to tackle ecological questions (Sueur and Farina, 2015). So far, the acoustics of animal species inhabiting freshwater habitats has been largely neglected probably because of the lack of flagship species or emblematic habitats. However, pioneer entomological studies suggested that several aquatic insect species could produce sound (Aiken, 1985) and recent conservation research determined ponds as high concern for biodiversity conservation (Verberk et al., 2006). Here, using a long-term passive acoustic monitoring approach, we (i) identified a total of 48 sound types differing in occurrence between the ponds, (ii) revealed a 24h cycle of acoustic activity differing among the ponds, and (iii) suggested that the use of AR as an index for automatic monitoring was limited by the need for SNR estimation.

Due to the lack of background research just mentioned above and in particular to the lack of an inventory clearly attributing sound types to species names, we were not able to identify the emitting species of the sound types we inventoried. Only a very small portion of the sound types identified were generated by terrestrial animals (4 sound types were identified as birds) but were rather faint. This probably results from the difference in impedance between air and water making it difficult for sound generated in air to transmit through water. The number of sound types, here named sound richness, is therefore probably an overestimation of the number of singing species as we were not able to assess the intra-specific-diversity and some sound types could also result from plant respiration that generates sonorous air bubbles (Felisberto et al., 2015, CD personal observations) or from terrestrial animal such as birds.

Even if sound richness could not be directly linked to a number of species, it represents an original facet of biodiversity that can be studied for its own. The three ponds showed different levels and dynamics of sound richness as illustrated with rarefaction curves commonly used to assess sampling effort in biodiversity studies (Gotelli and Colwell, 2011).

The number of recordings appeared sufficient to capture the sound richness in the ponds 2 and 3 as the rarefaction curves showed a plateau. Conversely, the rarefaction curve of sound types did not reach a plateau for pond 1 which had the highest sound richness. This suggests that pond 1 embedded a richer and more dynamic acoustic community with a higher diversity of sound types than in the two other ponds.

The distribution of the sound types among the three ponds was different enough to clearly pull apart the three ponds through a correspondence analysis indicating different acoustic communities. This multivariate analysis also revealed a higher heterogeneity of the points in the pond 3 than in pond 1 and 2. The Generalized Linear Mixed model confirmed the differences in sound richness among ponds and revealed as well differences along time, the sound richness of each pond evolving in a different way along the 24h cycle. All together, these results suggest that the three ponds harbour three different communities in terms of richness, composition, and abundance. These differences are in accordance with the three distinct ecological conditions we deliberately chose (open, semi-closed, closed habitats).

The signal to noise ratio (SNR) measures the ratio of the amplitude of the signal of interest over the amplitude of the surrounding noise. It is difficult to have reference values as it depends on several parameters such as surrounding noise level, amplitude of the source, distance from the receiver to the sources, obstacles between the source and the receiver or physical parameters of the matter in which the wave travels (*e.g.* humidity, temperature, viscosity). Compared to values in a terrestrial habitats which are usually comprised between 15 and 25 dB (Dabelsteen and Mathevon, 2002), the SNR was rather low here. A high proportion of recordings had a SNR of 0 dB meaning that they only contained signals which are less or as intense as the background noise. It is now rather difficult to identify whether these low SNR values were due to soft signals and/or loud background noise. The acoustic properties of ponds are unfortunately poorly known (Aiken, 1982; Forrest et al., 1993) compared to marine environments (Buckingham, 1992). These complex and heterogeneous environments may have very peculiar sound propagation patterns impacting the quality of the recordings, in particular the SNR. Further studies should therefore find solutions to increase the SNR such as removing the background noise with lossless filtering techniques.

Acoustic diversity can be estimated through the identification and count of sound types or species-specific songs. Even if very informative, this approach can be very time demanding when handling large sampling covering hours of audio recordings. Recently, acoustic indices have been developed to get a preliminary estimation of the acoustic diversity without sound or species labelling. We therefore tested five alpha acoustic diversity indices. The correlations between the acoustic indices and the aural analysis showed that the entropy based indices H_t and H_f were negatively correlated with the SNR, the richness and the abundance. This confirms that these metrics are very sensitive to background noise and may function in the reverse way as expected with simulations (Sueur et al., 2008b), as it was already pointed out for bird communities (Depraetere et al., 2012; Gasc et al., 2015). Conversely the envelope energy M, the number of major peaks of the mean frequency spectrum NP, and the Acoustic Complexity Index ACI were significantly positively correlated with richness and abundance. Although these three indices have been designed in the aim of circumventing the potential bias induced by the presence of noise and the absence of signals (Depraetere et al., 2012; Gasc et al., 2013), they were here also significantly positively associated with the SNR. Altogether our results show that the SNR could be an important confounding and misleading variable for these five indices and that these indices should be used when recordings have been performed in habitats selected for their low noise level, avoiding flowing water such as streams or waterfalls. The AR index was the single index correlated with neither richness nor abundance and the only index showing a positive and significant correlation with richness and abundance when taking SNR as a control variable in a partial correlation. The index AR is therefore a good candidate for revealing acoustic diversities within ponds. One of the major drawback of this index is that its ranking property makes independent studies hard to compare. Moreover our results show that it would be necessary to assess automatically the SNR to use AR. To compute the SNR without any manual identification of the signal and noise section within each recording is a technical challenge we could not solve preventing the use of the AR index on the complete set of recordings. Development in signal analysis is therefore still required to be able to monitor automatically pond acoustic diversity.

This preliminary study reveals that ponds we sampled were not silent habitats and that each pond revealed different acoustic diversity. New biodiversity programs should be developed to describe and understand the sound diversity of ponds. Efforts should be achieved through a species-specific approach based on behavioural and systematic sciences to identify the sound sources and, at the same time, through a community approach based on ecological sciences to allow rapid biodiversity assessment. Combining these two research routes should lead to a better knowledge of this still unknown facet of animal diversity.

2.6 Acknowledgements

We thank the Parc Naturel Régional de la Haute Vallée de Chevreuse (France) and the Office national de la Chasse et de la Faune Sauvage (St Benoist, Auffargis, France) for their help. We thank Pierre-Yves Martel and Arnaud Catherine for their help. We are grateful to Roger Mundry (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany) for providing R functions to study GLMM stability and to Bruno Naylor (Université Paris 13, Paris, France) for reviewing the English. We are grateful to Mitchell Aide and Klaus Riede for their helpful reviews.

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Chapter 3 :

Linking sound and ecology: acoustic community composition and lateral connectivity in a riverine floodplain

Lône Moiroud, Chanaz (Savoie, 73), May 2014

3.1 Summary

Context Freshwater acoustic communities differ spatially and temporally in relation to different habitats. Can the structure of the acoustic communities be linked to specific environmental variables ?

Problematics

- What are the underwater acoustic communities of floodplain channels ?
- Is the composition of these underwater acoustic communities related to any environmental variable ?
- What are the ecological processes resulting in the observed acoustic communities ?

Methods Long term acoustic monitoring along with temperature in six secondary channels of the Rhône river, differing by the lateral connectivity to the main river channel:

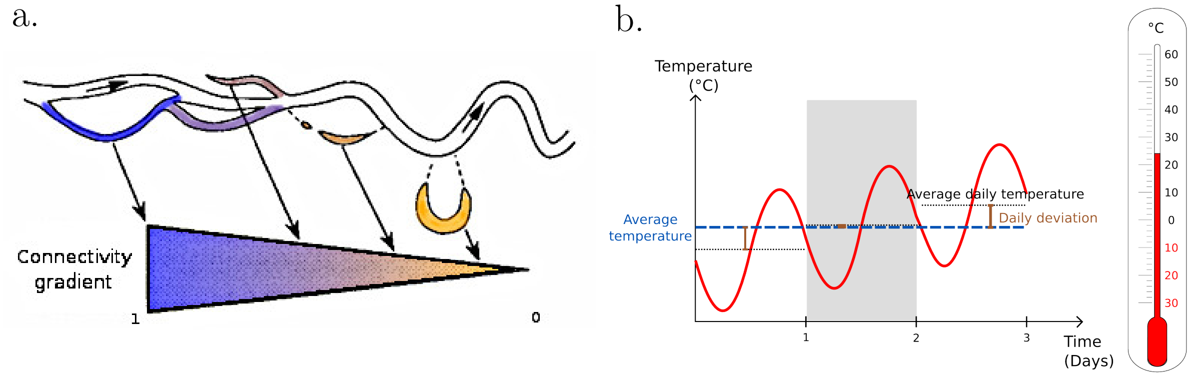


Figure 3.1 – **Studied environmental variables**. a. Lateral connectivity index of secondary channels in a riverine floodplain, this index varies from 0 (fully disconnected) to 1 (fully connected and flowing; after Paillex et al., 2013), b. Two temperature variables taken into account: the site temperature (mean temperature over the study period for each site) and the daily deviation of temperature (subtraction of the average site temperature per day to the average site temperature).

The six site were located on the lowest half of the connectivity gradient (*e.g.* sites that were flowing quite rarely and standing most of the time).

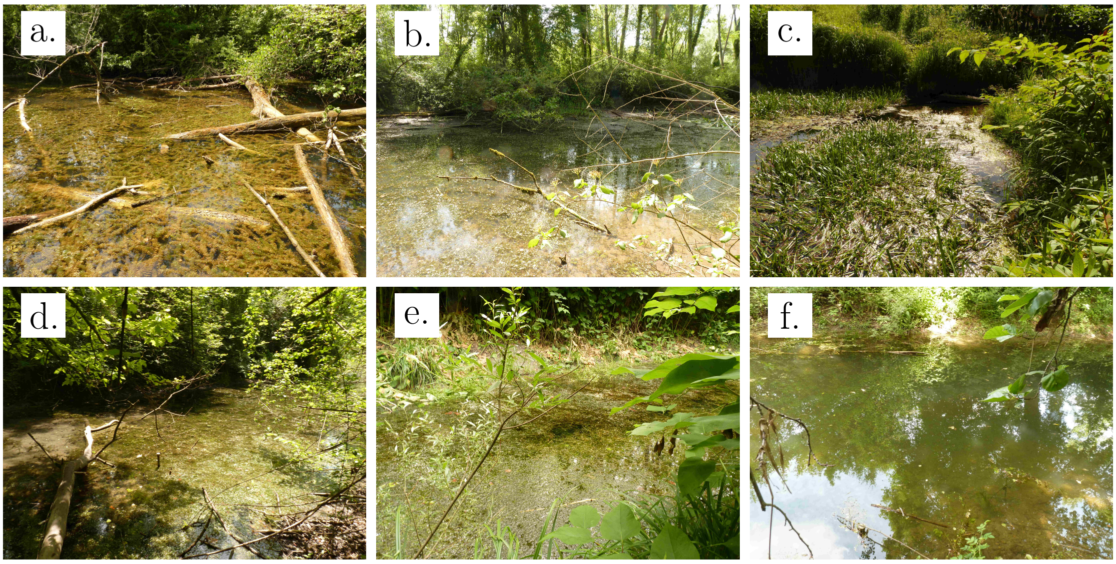
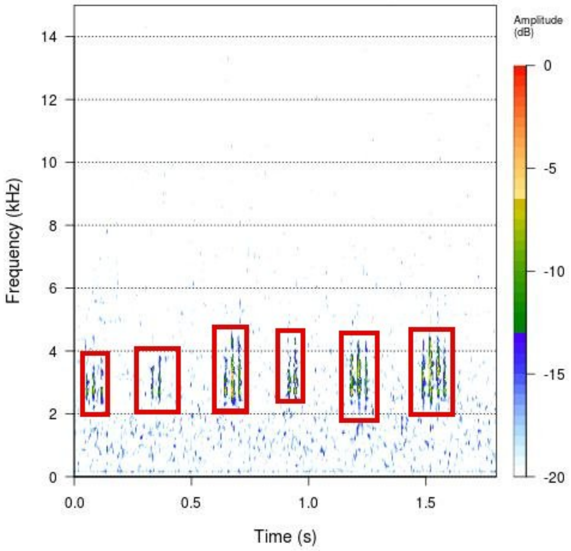


Figure 3.2 – **Pictures of the six sites**. The number in brackets indicate the value of lateral connectivity of the site. a. VILO (0.04), b. MORT (0.15), c. BEAR (0.20), d. ROSS (0.32), e. MOIR (0.50), f. GRAN (0.77)



- Assessment of the composition of the acoustic communities
- Measure of acoustic characteristics of acoustic communities
- Between correspondence analysis (bCA) to compare the composition of acoustic communities
- GLMM to investigate the link between acoustic composition and environmental variables

Figure 3.3 – **Example of visual identification of sound types composing the acoustic communities**. Each sound event was annotated and allocated to a sound type.

- bCA on the composition macro-invertebrate communities to compare the composition of macro-invertebrate communities
- Study of acoustic characteristics in relation to connectivity
- Permutation test to study the level of frequency dispersion of acoustic communities

Main results

- 128 different sound types differing by their acoustic characteristics
- The six secondary channels host distinct acoustic communities
- Significant relationships between the first and third bCA axes performed on acoustic communities and lateral connectivity
- Relationship between macro-invertebrate community composition and lateral connectivity
- Relationship between site acoustic characteristics and connectivity
- Acoustic characteristics of communities more dispersed than expected by chance

Perspectives These results open theoretical perspectives on the processes structuring acoustic communities which may be related to sound propagation in different environments or acoustic partitioning. The relationship between acoustic community composition and an environmental variable also suggests that acoustics could be a useful tool to monitor freshwater environments.

The preliminary exploration of processes structuring acoustic communities suggests that propagation properties of the six secondary channels are different. Moreover the permutation results indicate that an acoustic partitioning of the acoustic space takes place in those environments.

Related communications and publications

- Linking sound and ecology: acoustic community composition and lateral connectivity in a riverine floodplain. **Camille Desjonqueres**, Fanny Rybak, Emmanuel Castella, Diego Llusia, Jérôme Sueur. Manuscript in review in *Oecologia.*
- Acoustic diversity in freshwater habitats: the effect of lateral connectivity in a riverine floodplain. **Camille Desjonqueres**, Fanny Rybak, Emmanuel Castella, Diego Llusia, Jérôme Sueur. 2015. Oral presentation at the International Bioacoustics congress, Murnau, Germany.

The first section corresponds to the article in review in *Oecologia*, the second section presents preliminary tests of the potential processes acting in freshwater environments.

3.2 Article

3.2.1 Introduction

Various animals produce sound during communication, sharing information on their identity, location, physiological and behavioural state or environment (Bradbury and Vehrencamp, 1998). These signals are the heart of bioacoustics, a discipline which mainly aims at deciphering the modalities and contexts of animal communication by understanding the emission, propagation, and reception of sounds used to exchange information (Fletcher, 2007). If such sounds are by essence a unique material to study animal behaviour, they also appear to bear some information on the ecology of populations, communities, and landscapes. Listening to animal sounds in an ecological framework is the main perspective of ecoacoustics, a newly emerged discipline (Sueur and Farina, 2015). The ecoacoustics paradigm consists in using all sounds emanating from environments to monitor, describe, and study biodiversity in order to tackle fundamental and applied ecological questions such as climate change (Krause and Farina, 2016). As such, ecoacoustics derives from bioacoustics but scales up from the individual unit to the population, community, and/or landscape units to establish a link between sound and ecology.

According to ecoacoustics, a collection of sounds produced by a set of organisms coexisting in a given habitat over a specified time and sharing the same acoustic space constitutes an acoustic community (Gasc et al., 2013). The structure of an acoustic community depends on the communication signals and the by-product sounds due to other behaviours such as feeding, predation, and navigation. The occurrence of all these sounds in the environment is directly determined by the presence and activity of the emitters, themselves conditioned by favourable species-specific environmental resources and species-specific physiological tolerance: a potential emitter is acoustically active only if appropriate conditions are met (Llusia et al., 2013). Sound properties, such as amplitude, repetition rate or frequency content, are also remarkably affected by environmental variables. Temperature influences almost all parameters of the sounds produced by ectothermic organisms including macro-invertebrates (Sanborn, 2005). In addition to these proximate factors shaping the structure of the acoustic community, the ultimate factors need to be carefully considered as well.

In community ecology, the structure of any community is thought to be determined by a combination of two families of processes: (1) stochastic processes, such as chance colonization, random extinction, and ecological drift, and (2) deterministic processes related to the concept of ecological niche (Chase and Myers, 2011). The deterministic processes refer to two main evolutionary factors: interactions between species and environmental filtering (Morin, 2011). While the stochastic processes at the origin of acoustic communities have never been considered yet, the deterministic processes have been formalized under two theories: the acoustic niche hypothesis and the acoustic adaptation hypothesis. On the one hand, the role of inter-specific interactions is covered by the acoustic niche hypothesis or ANH (Krause, 1987), directly inspired from the Hutchinsonian niche concept (Hutchinson, 1957), in which the resource shared by species and individuals is the acoustic space. The ANH is based on the assumption that species vocalizing at the same time in a location tend to avoid time and/or frequency overlap to reduce the cost of masking. This assumption should lead to the observation of an over-dispersion of sound features and therefore to a partitioning of the acoustic space. On the other hand, the environmental filtering hypothesis can be directly linked to the acoustic adaptation hypothesis, or AAH. AAH stipulates that the acoustic properties of habitats, mainly determined by ground morphology, ambient noise and plant structure and density, have shaped animal sounds, resulting in the maximization of their propagation (Morton, 1975). The environment would thus act as an evolutionary filter on the properties of the signal and would lead to a convergence of sounds towards an optimum of transmission. These two debated hypotheses – driving the properties of sounds in opposite directions – are often referred to to explain dissimilarities observed within and between communities as illustrated within Pacific and Mediterranean landscapes (Gasc et al., 2013; Bormpoudakis et al., 2013). The ANH and the AAH cover the crossed-effects of several ecological factors related to the environment, such as ambient temperature, relative humidity, or structure of the habitat and to the intra- and inter-specific interactions including acoustic masking and collective calling behaviour through chorusing.

In freshwater habitats, the diversity and composition of macro-invertebrate communities are traditionally estimated to assess the ecological quality of habitats due to the sensitivity of these organisms to disturbing factors such as chemical pollution or temperature changes (Oertli et al., 2005). Macro-invertebrate includes the largest number of soniferous species in freshwater environments (Aiken, 1985). Water beetles (Coleoptera), water bugs (Hemiptera), and caddisflies (Trichoptera) are indeed known to emit sounds underwater, mostly to communicate (Aiken, 1985). These taxa are therefore likely to constitute a large fraction of sound sources in freshwater environments as recently testified in temperate ponds (Desjonquères et al., 2015). Contrary to terrestrial and marine acoustic communities which have been the focus of several ecoacoustic studies (*e.g.* Parks et al., 2014; Duarte et al., 2015), freshwater acoustic communities have rarely been investigated and are thus poorly understood.

Among freshwater habitats, European riverine floodplains are highly dynamic environments that have been largely modified by anthropic actions (Dudgeon et al., 2006). The main changes operated being embankments, dams and by-pass canals (Lamouroux et al., 2015). The river Rhône is no exception to this general European and even worldwide trend with about a third of its course (162 km out of 522 km) being artificial channels for hydro-power plants (Lamouroux et al., 2015). These human infrastructures have severe effects on the physical and functional properties of the river. One of the main modified environmental factors is the minimum water discharge that can be significantly modified, with reductions from its natural discharge of up to several orders of magnitude (*e.g.*, 1000 $m^3.s^{-1}$ to 10 $m^3.s^{-1}$ in Lyon, France; Lamouroux et al., 2015). Floodplain channels, which are shaped by flood disturbances, are among the most impacted habitats in freshwater environments (Castella et al., 2015; Paillex et al., 2015). Lateral connectivity is an environmental variable quantifying the level of connection of the floodplain channels to the main river. Lateral connectivity varies from high values in fully connected channels flowing all year round to low values in fully disconnected sites. In between these two extremes, channels covering the whole spectrum of connection to the main river – from high to low flow and from connected by yearly floods to connected only by centennial floods – can be found. Macro-invertebrate and fish communities were found to be ordinated along lateral connectivity (Castella et al., 2015; Paillex et al., 2015; Lamouroux and Olivier, 2015).

In this study, we explored acoustic diversity in freshwater habitats and its potential link with key ecological features of these environments. With a continuous passive acoustic monitoring at six floodplain channels of the river Rhône, we surveyed and described for the first time underwater acoustic communities of floodplain channels focusing on the Rhône case study and searched for potential environmental determinants of their composition. Using non-invasive ecoacoustic techniques, we tested whether the acoustic community diversity was site-specific and influenced by water temperature and lateral connectivity, two environmental variables previously shown to regulate the composition of macro-invertebrate communities.

3.2.2 Materials and methods

3.2.2.1 Study sites

Passive acoustic monitoring of freshwater communities was carried out in six secondary channels located in two reaches (Belley and Brégnier-Cordon) of the French Upper Rhône floodplain (Figure 3.4). These six sites (hereafter referred to as BEAR, GRAN, MOIR, MORT, ROSS, and VILO) were chosen to account for different lateral connectivity levels (see section Environmental variables) among a set of 44 sites studied in the restoration program of the Rhône (Lamouroux et al., 2015). The distance between sites was 14.46 ± 10.73 km (mean ± sd).

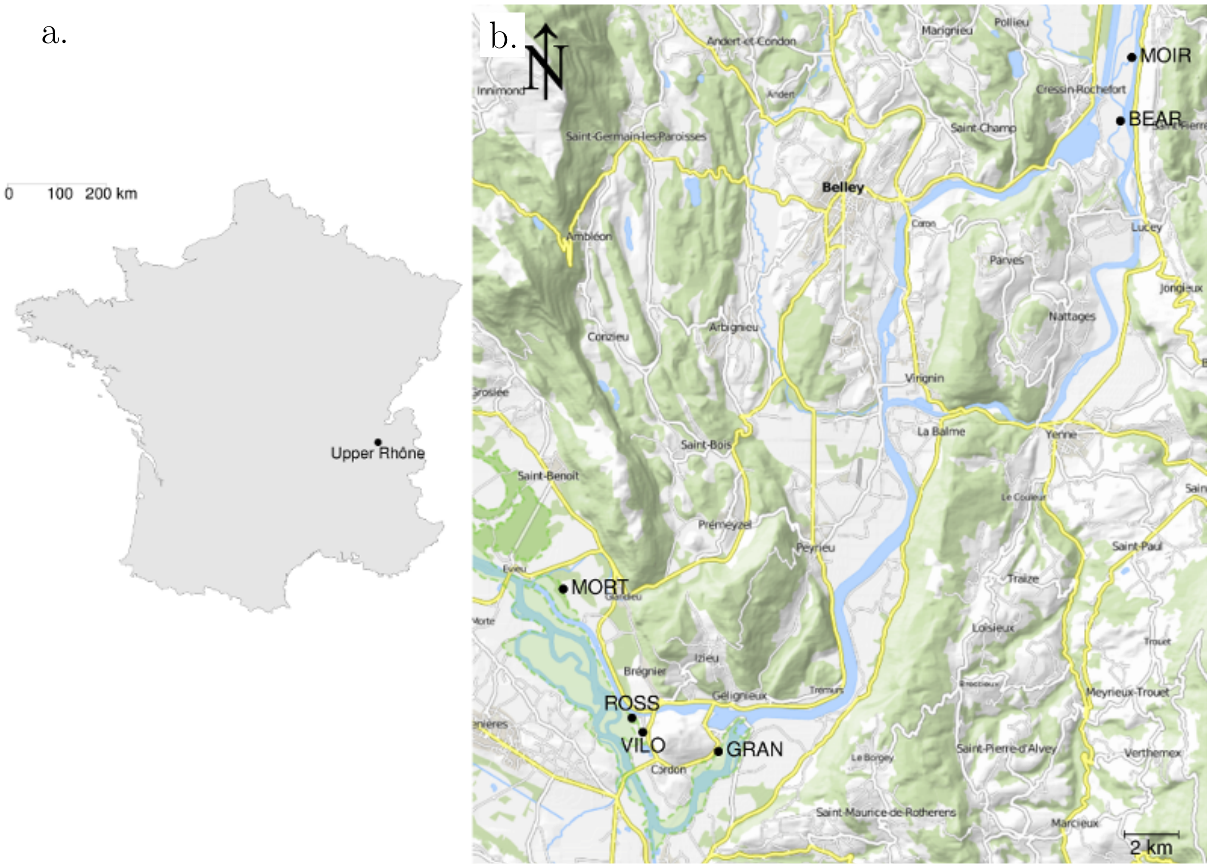


Figure 3.4 – **Map showing the location of the study sites.** General localisation in French mainland (a) and local position of the sites(b).

3.2.2.2 Acoustic monitoring

To record the sounds emanating from each site, the acoustic communities were monitored with an autonomous recording platform consisting of two hydrophones HTI-96 (flat frequency response between 20 Hz and 40 kHz) connected with a 20 m cable to a single digital audio field recorder SM2 (Wildlife Acoustics, 2009). The SM2 recorders were set up to record uncompressed .wav audio files at a 44.1 kHz sampling frequency and a 16 bit digitization depth. The two hydrophones were placed 6.3 ± 2.1 m away from each other at each site and were attached underwater to a stake at 0.18 ± 0.07 m above the sediment, with their piezoelectric element directed downward toward the sediment. The recording schedule was set to 1 min per hour, 24 hours a day. The acoustic monitoring lasted 15 days, from the 20th of June 2014 to the 4th of July 2014, resulting in 4,320 one-minute audio files. To avoid weather disturbances, such as rain or wind, that could impair acoustic analyses, five days of recordings with similar stable weather conditions were selected across the study period (i.e., 20/06/2014, 22/06/2014, 26/06/2014, 01/07/2014 and 04/07/2014) for further analyses. These five days, that resulted in a subset of 1,440 one-minute files (6 sites x 2 hydrophones x 24 hours x 5 days), were selected based on wind speed and rainfall measurements collected at two weather stations from the Réseau d'Observation Météo du Massif Alpin (ROMMA, http://www.romma.fr/) located in Brégnier-Cordon (45°38'05"N, 05°37'13"E) and Chrindrieux (45° 49'18"N, 05°51'05"E).

The amplitude of water level variation during the sampling period was low ranging from -4 mm and 25 mm around the mean level during the study period.

3.2.2.3 Assessment of the composition of the acoustic communities

The subset of 1,440 one-minute files was analysed in a random order by aural listening and visual inspection of oscillograms and spectrograms with the audio software Audacity (version 2.0.5). This analysis focused on the detection of sound events, *i.e.* any substantial shift in sound amplitude over background noise showing a singular acoustic structure, and hence expected to be produced by freshwater species or other biotic sources. Since no sound reference exists for most freshwater species (except anurans), species identification was not conducted preventing a direct link between a particular sound event and a species. Each sound event in each recording was time delimited and allocated to a specific sound type. A sound type was defined as a group of similar sound events characterized by their temporal and spectral properties (sound duration, dominant frequency, frequency modulation). This resulted in a presence-absence matrix of sound types across the recordings determining the sound type composition of each recording.

3.2.2.4 Acoustic characteristics of freshwater acoustic communities

To characterize the unknown acoustic communities found in the studied floodplain channels, all the sound types were described by measuring the acoustic properties of a random subset of sound events (n=1–6). For each selected sound event, the dominant frequency and duration were measured using Audacity with a 12 Hz and 1 ms precision respectively. Additionally, each sound type was allocated to one of the seven following categories: (1) pure tone: continuous sound lasting more than 0.1 s with a frequency band narrower than 500 Hz; (2) noisy sound: continuous sound lasting more than 0.1 s with a frequency band broader than 500 Hz; (3) simple pulse: sound lasting less than 0.1 s; (4) composed pulse: sound composed of several simple pulses; (5) harmonic sound: continuous sound with harmonics; (6) irregular sound: sound without a clear pattern; and (7) composed sound: sound composed of at least two of the previous categories.

3.2.2.5 Comparison of the composition of acoustic communities

To compare the composition of the acoustic communities among the six studied floodplain channels, an hourly presence-absence matrix of sound types, composed of 1,440 rows (number of files) and 128 columns (number of sound types) was first created. The information provided by the two hydrophones within each site was pooled together. This database was grouped daily by transforming the hourly presence-absence matrix into a daily presence-absence matrix of sound types composed of 30 rows (5 days x 6 sites).

The results of a Correspondence Analysis (CA) performed on this daily matrix were processed with a between-class Correspondence Analysis (bCA) using sites as a factor of variance maximization. The first three axes of the bCA were used to: (1) visualize the sound type composition differences between sites; (2) identify the sound types driving these differences; and (3) study the relationship between the acoustic composition and the environmental variables.

3.2.2.6 Environmental variables

To test whether the composition of the acoustic communities was related to the main environmental variables, water temperature and lateral connectivity were estimated at each site.

A water temperature sensor (Onset Tidbit v2) was attached to a submerged stake next to each hydrophone. The 12 sensors recorded water temperature every hour in phase with the acoustic recordings. The hourly temperature was extracted for the five selected days. Two variables for temperature were computed to disentangle the intra and inter-site variation of temperature. The site temperature was calculated as the mean temperature over the study period for each site in order to assess the inter-site variation of temperature.

The daily deviation of temperature was then calculated to assess the intra-site variation of temperature by subtracting the average site temperature per day from the average site temperature.

Indirect measures of lateral connectivity were introduced in previous studies to reduce the cost of monitoring year-round the connection of each site to the main river and the drag forces applied to the sites (Paillex et al., 2007). Specifically, lateral connectivity was estimated with the index described in Paillex et al. (2007). This connectivity index was shown to be a suitable proxy of connection frequency and flood disturbance regime in the study channels (Riquier et al., 2015). The calculation of the index is based on four environmental variables: (i) the organic matter content of the top 5 cm of the sediment, measured by weight loss on ignition; (ii) the electrical conductivity (μSiemens.cm^{-1}) of the water; (iii) the dimensionless Simpson diversity of the mineral sediment composition calculated over four categories (clay + silt/sand/gravels/pebbles); and (iv) the horizontal cover by submerged vegetation. The four variables measured for all the 44 sites of the restoration program and for all the sampling years were processed in a standardized PCA. The index of connectivity was made up with the scores of the sites on the first axis of the PCA scaled between 0 and 1 (lowest and highest connectivity respectively, Paillex et al., 2007).

3.2.2.7 Link between acoustic composition and environmental variables

A set of three Generalized Linear Mixed Models (GLMM), with Gaussian error structure and identity link function, was used to analyse the relationship between the acoustic composition of the sites and the environmental variables. The first three bCA axes were used as the response variable in the models. Average site temperature, daily deviation in temperature per site, and lateral connectivity were included as fixed effects, and site and date as random effects. To keep type I error at the nominal level of 5%, all required random slopes were also included (Barr et al., 2013). Site temperature, daily temperature, and lateral connectivity were approximately symmetrically distributed. The environmental variables were z-transformed (mean of zero and a standard deviation of one) to reduce the chance of obtaining a non-converging model. The model was fitted in R (R Core Team, 2015) using the function lmer of the R-package lme4 (Bates et al., 2014, version 1.1.10). The assumptions of normality and homogeneity of the residuals were checked by visually inspecting a quantile-quantile plot and the residuals against the fitted values, both indicating no deviation from these assumptions. Model stability was checked by excluding data points one at a time from the data. Variance inflation factors (Field, 2009) were derived using the function vif of the R-package car (Fox and Weisberg, 2011, version 2.1.0) applied to a standard linear model excluding the random effects and did not indicate collinearity between fixed effects to be an issue. The full model was compared with the null model (*e.g.* excluding all the predictors or the predictor tested) to test the model and predictor significance.

3.2.3 Results

3.2.3.1 Acoustic characteristics of freshwater acoustic communities

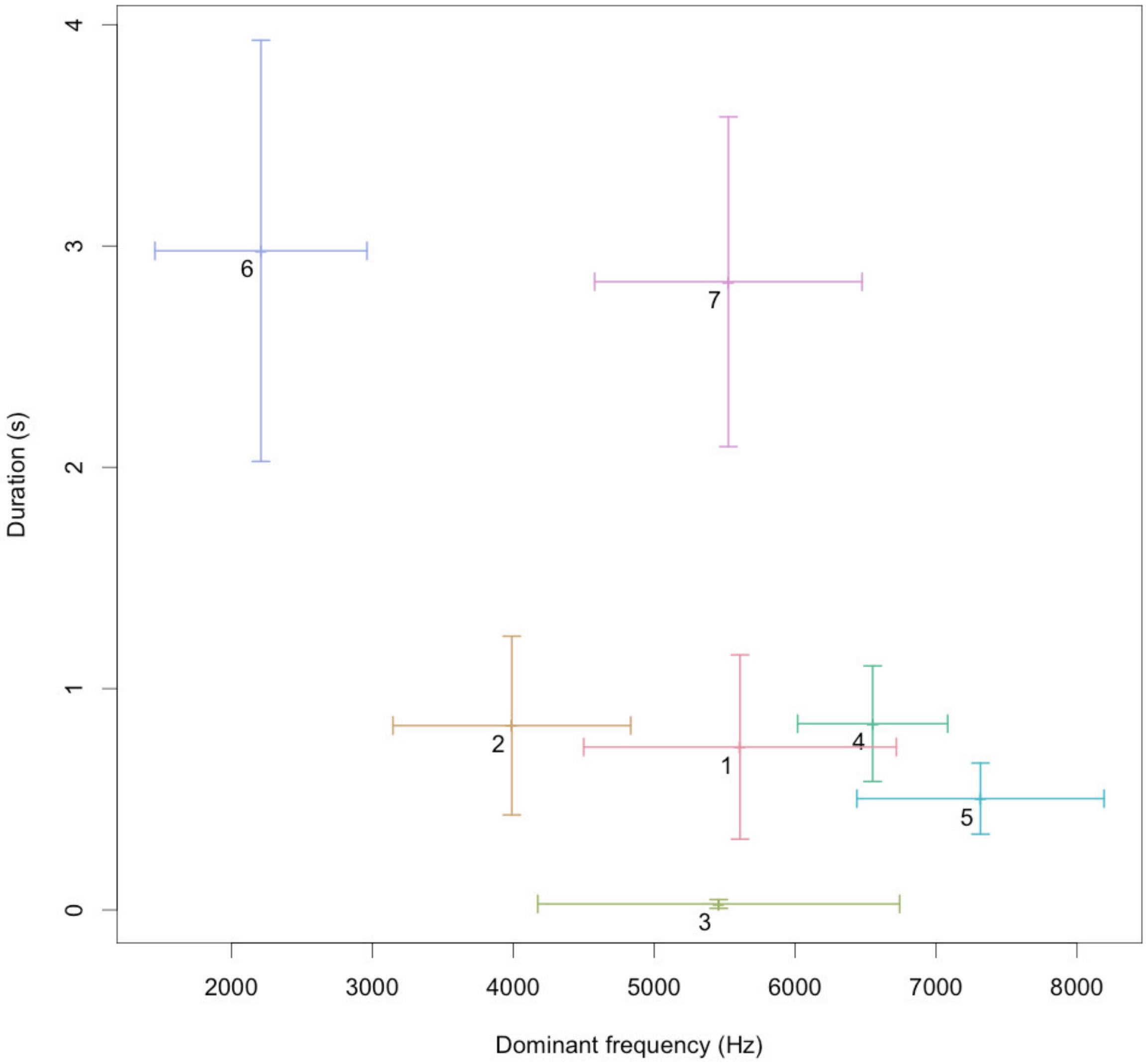


Figure 3.5 – **Main properties of the seven sound type categories.** The dispersion of sound dominant frequency (Hz) and duration (s) are shown with 95% confidence interval segments. The intersection of the segments corresponds to the mean. Sample sizes: 54, 109, 37, 205, 85, 71, and 48 for the categories from 1 to 7, respectively. See Table 3.1 for category description.

Table 3.1 – **Characteristics and abundances of the seven categories of sound types**: number of sound types in each category, average number of times a sound type occurs in each category, dominant frequency and duration given as mean ± s.d. Categories refer to: (1) *pure tone*: continuous sound lasting more than 0.1 s with a frequency band narrower than 500 Hz; (2) *noisy sound*: continuous sound lasting more than 0.1 s with a frequency band broader than 500 Hz; (3) *simple pulse*: sound lasting less than 0.1 s; (4) *composed pulse*: sound composed of several simple pulses; (5) *harmonic sound*: continuous sound with harmonics; (6) *irregular sound*: sound without a clear pattern throughout; and (7) *composed sound*: sound composed of at least two of the previous categories.

Category	**Number of sound types**	**Average occurrence**	**Dominant frequency (Hz)**	**Duration (s)**
1. Pure tone	12	5.7	5608 (± 4158)	0.74 (± 1.56)
2. Noisy sound	24	20.2	3989 (± 4493)	0.83 (± 2.15)
3. Simple pulse	7	312.9	5457 (± 3985)	0.03 (± 0.06)
4. Composed pulse	45	13.4	6549 (± 3889)	0.84 (± 1.91)
5. Harmonic sound	18	19.4	7314 (± 4125)	0.50 (± 0.76)
6. Irregular sound	12	373.4	2210 (± 3233)	2.98 (± 4.09)
7. Composed sound	10	5.0	5526 (± 3353)	2.84 (± 2.63)
Total	128	64.3	5462 (± 4247)	1.14 (± 2.37)

A total of 128 sound types were identified (Table 6.2). The sound types had a mean duration of 1.14 ± 2.37 s (Table 3.1) and an average dominant frequency of 5.46 ± 4.25 kHz (Table 3.1). Half of the sound types had their dominant frequency between 2.3 and 8.8 kHz. The seven categories of sound types were characterized by different diversity and abundance (Table 3.1) as well as different duration and frequency characteristics (Figure 3.5, Figure 3.6). The category of composed pulses was the most diverse (45 sound types) across the studied acoustic communities, whereas the simple pulses category was the least diverse (7 sound types). Irregular sounds and simple pulses were the most commonly recorded categories, whereas composed sounds and pure tones were the least abundant. Simple pulses had the shortest average duration (0.027 ± 0.061 s) and irregular sounds the longest (2.978 ± 4.091 s). Irregular sounds had the lowest average dominant frequency (2210 ± 3233 Hz) and harmonic sounds the highest (7314 ± 4124 Hz).

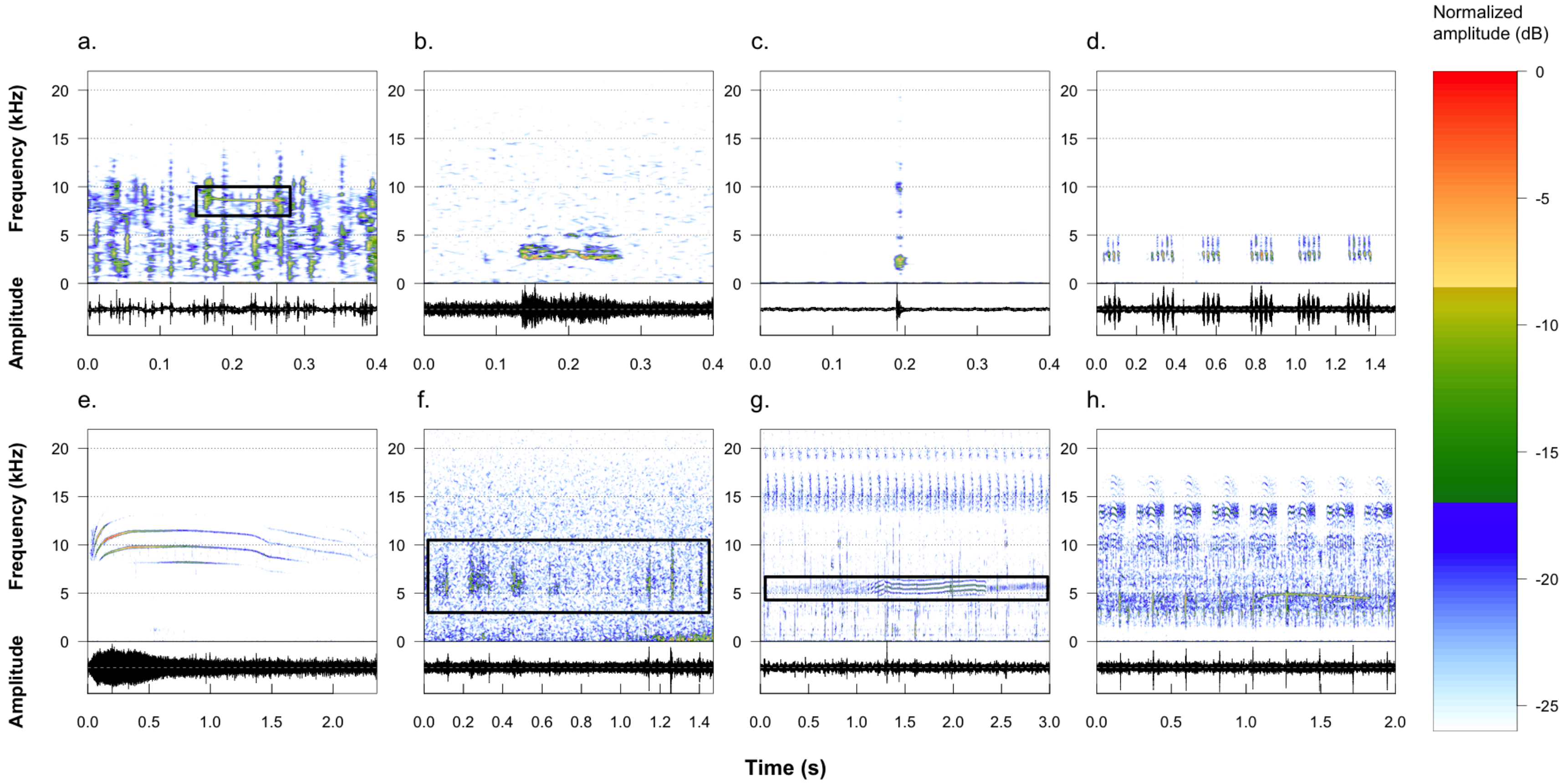


Figure 3.6 – **Spectrograms and oscillograms of an example of each of the seven sound categories and of one recording containing several categories** (Fourier window length: 512 samples, frame overlap: 50%, window type: Hanning): a. sound type 104, *pure tone*; b. sound type 103, *noisy sound*; c. sound type 75, *simple pulse*; d. sound type 1, *composed pulses*; e. sound type 50, *harmonic sound*; f. sound type 63, *irregular sound*; g. sound type 118, *irregular sound*; and h. recording from MORT on the 26th of June at 12:00 am.

3.2.3.2 Comparison of the composition of acoustic communities

The freshwater acoustic communities were characterized by a high variability in sound types, showing a site-specific acoustic composition. Only nineteen sound types (15%) were found in all the studied sites. An average of 29 ± 8 different sound types where found per day in each site.

The bCA of the composition matrix revealed a significant difference in sound type composition between the sites (permutation test: 1000 permutations, p-value<0.001). The first three axes explained 73.3% of the overall variance (first axis: 29.4%, second axis: 22.9%, third axis: 21.0%; Figure 3.7a-b). The coordinates of the sites in the three first bCA axes revealed BEAR as the most distant site from the other sites (Figure 3.7a-b). The ordination of the sites was best explained by the positive contributions of one composed sound (48), one composed pulse (56), and two pure tones (65 and 67) to the first axis, the positive contribution of three noisy sounds (4, 99, and 107) and three composed sounds (112, 118 and 128) to the second axis, and the negative contribution of a diverse group of sounds (76, 81, 83, 93, 101, 115, and 117) to the third axis (Figure 3.7c-d). Among these influential sound types, none were in the categories 3 (simple pulses) or 6 (irregular sounds), which had the highest average occurrence (Table 3.1).

3.2.3.3 Environmental characteristics of the sites

The mean water temperature differed significantly between the six sites, with MOIR being the coldest site (12.5 ± 0.7°C) and MORT the warmest site (19.7 ± 1.2°C; ANOVA: df= 1434, F=1410, p-value<0.001, Appendix 6.3, Table 6.3). The daily deviations from the average temperature ranged from -1.61°C to 0.89°C.

The first axis of the PCA, used to assess connectivity, explained 62.9% of the total variability. The sediment size diversity was positively correlated to the first axis and vegetation cover and the amount of organic matter in the sediment were negatively correlated to the first axis. The conductivity was not highly correlated with the first axis of the PCA (absolute value of 0.43 while the values for the other variables ranged from 0.85 to 0.91). The order of increasing lateral connectivity of the sites was VILO, MORT, BEAR, ROSS, MOIR, GRAN (Appendix 6.3, Table 6.3).

3.2.3.4 Link between acoustic composition and environmental variables

The sound type composition explained by the first and second bCA axes was not linked to any of the environmental variables, as shown by the GLMMs testing the acoustic composition in relation with average site temperature, and daily temperature deviation, and lateral connectivity (overall model significance for the first bCA axis: df=3, χ^2=2.19, p-value=0.53; and for the second bCA axis: df=3, χ^2=0.27, p-value=0.96; Table 3.2). In

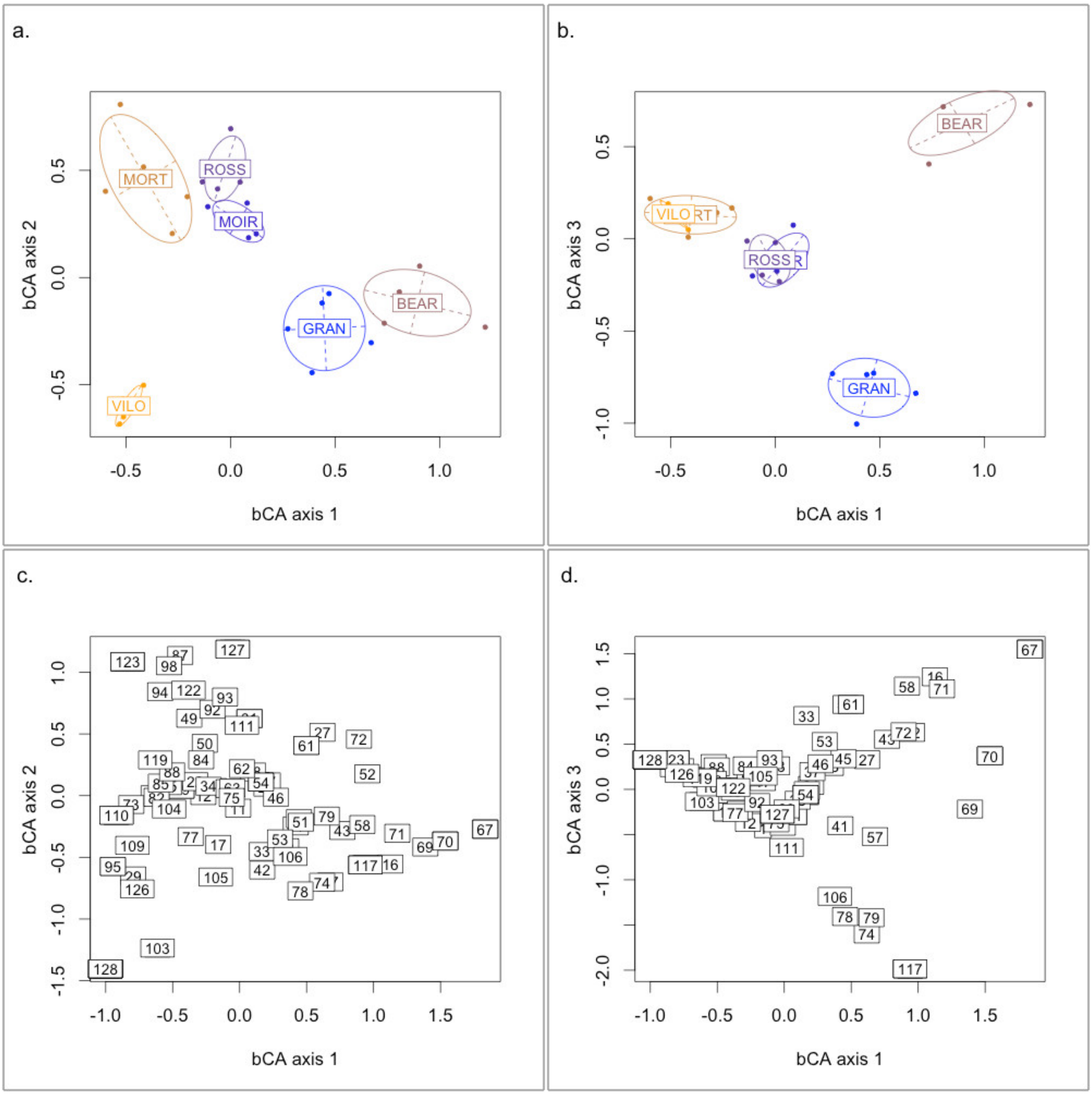


Figure 3.7 – **Between Correspondence Analysis (bCA) applied to the composition of the acoustic communities**. The sites were used as factors for variance maximization. The plots a. and b. are projections of the composition of the acoustic communities on the first three axes of the bCA. Each point corresponds to the composition of the acoustic community recorded at one site during one day. The distance between points indicates acoustic composition dissimilarity. The dispersion ellipses surround the position of an acoustic community providing an index of the dispersion around the centroid (67% of the acoustic compositions are expected to be in the associated ellipse). The plots c. and d. are projections of the sound types according to the first three axes of the bCA. The labels refer to the number attributed to each of the 128 sound types.

contrast, the third bCA axis was significantly correlated with lateral connectivity (df=1, χ^2=10.20, p-value $<$ 0.01, Table 3.2).

An inspection of the models characteristics revealed a high random intercept for BEAR

Table 3.2 – **Results of the six GLMMs**. For each model and each term in the models, the estimate, the standard error, the χ^2, the number of degrees of freedom and the p-values are reported, except for intercepts (p-value *<0.05, **<0.01, ***<0.001). For statistical details, see subsection *Link between acoustic composition and environmental variables* of the Materials and Methods.

Response variable	**Term**	**Estimate error**	**Standard (χ^2)**	**Statistics of freedom**	**Degrees**	**P-value**
Axis 1						
	Intercept	0.07	0.16	$^{(1)}$	$^{(1)}$	$^{(1)}$
	Lateral connectivity	0.06	0.22	0.08	1	0.776
	Average temperature	-0.22	0.22	0.86	1	0.344
	Daily temperature	0.008	0.02	0.11	1	0.740
Axis 1 excluding BEAR	Intercept	-0.35	0.26	$^{(1)}$	$^{(1)}$	$^{(1)}$
	Lateral connectivity	0.34	0.04	14.74	1	0.0001 ***
	Average temperature	0.02	0.01	0.77	1	0.38
	Daily temperature	0.03	0.03	0.68	1	0.41
Axis 2						
	Intercept	-0.28	1.50	$^{(1)}$	$^{(1)}$	$^{(1)}$
	Lateral connectivity	0.07	0.22	0.09	1	0.76
	Average temperature	0.02	0.09	0.05	1	0.83
	Daily temperature	-0.02	0.04	0.16	1	0.69
Axis 2 excluding BEAR	Intercept	-0.08	1.82	$^{(1)}$	$^{(1)}$	$^{(1)}$
	Lateral connectivity	0.03	0.26	0.02	1	0.90
	Average temperature	0.009	0.11	0.008	1	0.93
	Daily temperature	-0.03	0.04	0.45	1	0.50
Axis 3						
	Intercept	0.02	0.07	$^{(1)}$	$^{(1)}$	$^{(1)}$
	Lateral connectivity	-0.50	0.09	10.20	1	0.001 **
	Average temperature	-0.19	0.09	2.12	1	0.15
	Daily temperature	-0.05	0.05	0.96	1	0.33
Axis 3 excluding BEAR	Intercept	-0.10	0.04	$^{(1)}$	$^{(1)}$	$^{(1)}$
	Lateral connectivity	-0.40	0.05	12.33	1	0.0004 ***
	Average temperature	-0.13	0.05	3.94	1	0.047 *
	Daily temperature	-0.02	0.02	0.29	1	0.59

(1) Not shown due to the lack of meaningful interpretation.

in model 1 and 3 (Appendix 6.3, Table 6.4). In addition, the inspection of the bCA space highlighted the outlier position of this site (Figure 3.7). Thus, when excluding BEAR, GLMMs identified highly significant relationships between the first and third bCA axes and lateral connectivity (df=1, χ^2=14.74, p-value<0.001, Figure 3.8a; df=1, χ^2=12.33, p-value<0.001, Figure 3.8b, respectively). This model also uncovered a relationship between the third bCA axis and average site temperature (df=1, χ^2=3.34, p-value<0.05). None of the environmental variables were associated to the second axis of the bCA (overall model significance: df=3, χ^2=0.49, p-value=0.92).

3.2.4 Discussion

The ecoacoustic approach showed that the composition of acoustic communities in six secondary channels of the Rhône floodplain could be characterized and distinguished with a rather reasonable sampling effort and without taxonomic expertise. The underwater acoustic survey conducted over 15 days revealed an important diversity across these communities, composed of 128 sound types within seven categories. This finding supports a previous study revealing a remarkable underwater acoustic diversity in temperate ponds (Desjonquères et al., 2015). The sound type categorization pointed out that the most diversified sounds were composed of several simple pulses, a temporal structure that is often found in the signals produced by aquatic insects and amphibians (Gerhardt and Huber, 2002), two important groups of soniferous animals inhabiting aquatic environments. The sound types the most often encountered were made of simple pulses and showed an irregular structure. These sounds were probably the by-product of animal movement and feeding behaviours. Moreover, the 128 sound types inventoried were not randomly distributed among the six monitored floodplain channels, but their distribution was site-specific as testified by the multi-variate analysis and by the low percentage of sound types (15 %) shared by all the sites. This acoustic diversity pattern suggests the occurrence of different freshwater communities with significant within- and between-variation of community composition. The drivers of this singular acoustic signatures can be sought in a series of proximate and ultimate factors hereafter considered successively.

First of all, the presence and the activity of soniferous ectotherms, terrestrial or aquatic, may be set by ambient temperature, each species occupying a determined thermal niche (Angilletta, 2009), since temperature limits the appropriate conditions for communicating with sound or, even, for surviving. Here, the within-site ambient temperature was rather stable, with variations ranging around 1.5°C during the study period, implying a restricted potential effect of temperature within each acoustic community at short-term. The substantial thermal differences observed between sites, with variation in average temperatures ranging around 7°C, were expected to contribute largely to the differences in

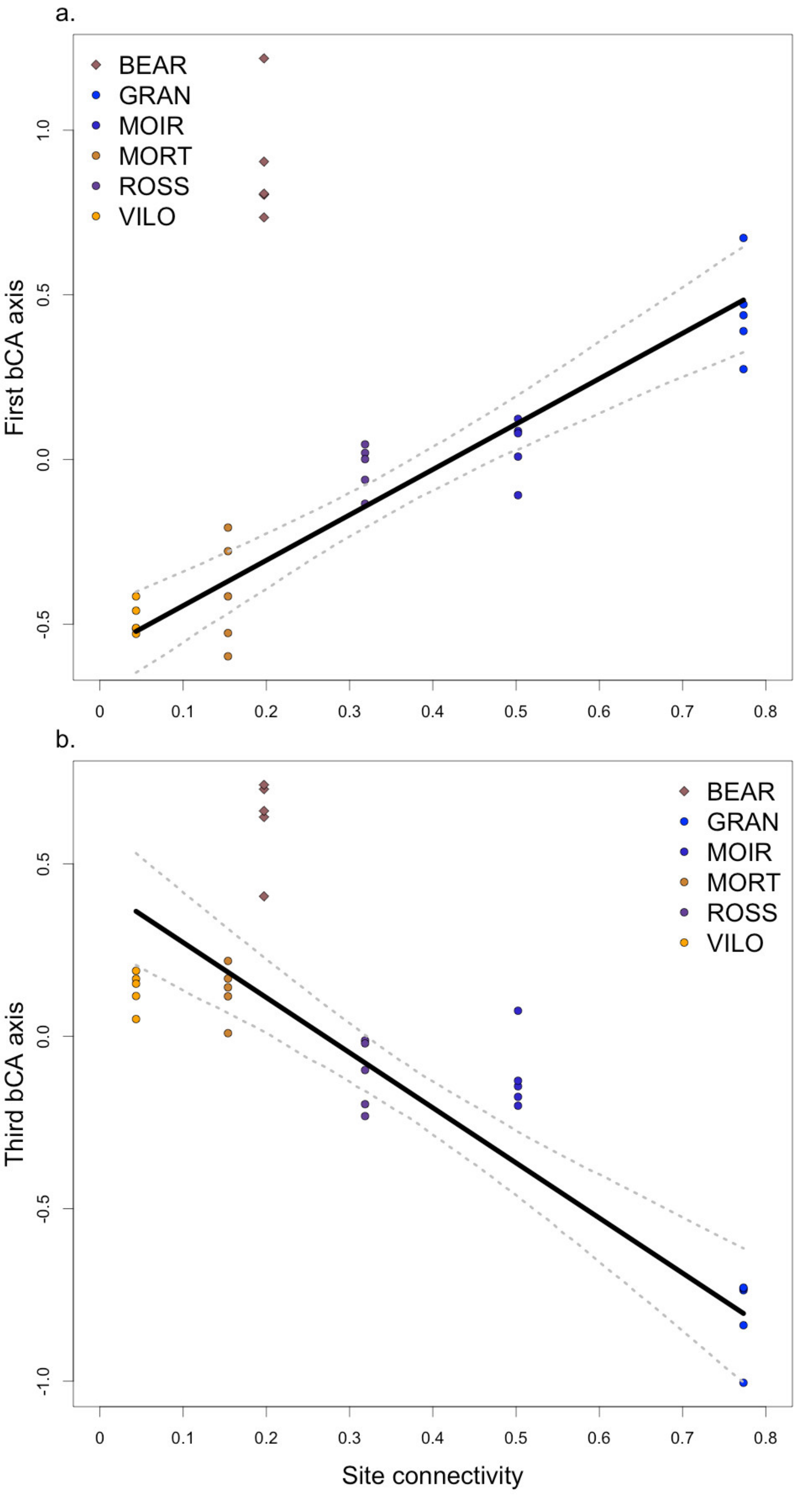


Figure 3.8 – **Relationship between the first a. and third b. bCA axes and lateral connectivity**. In both cases each point represents a day of recording in one site. The plain grey line shows the fitted model, excluding the site BEAR. The dotted lines are the 95% confidence interval.

composition between the acoustic communities. However, only the third axis of the multivariate analysis revealed an effect slightly supported with a p-value just below the 5% significance level. Therefore, the ambient temperature could not be identified as a key determinant of acoustic community patterns, a result that has so far not been tested on macro-invertebrate community patterns in the same floodplain.

Beyond ambient temperature, other environmental parameters might have a role in both within and between-community patterns. The acoustic adaptation hypothesis (AAH) suggests that the environment shapes the features of sound signals as a filter retaining only the signals adapted to the environment. According to the AAH, sites having similar propagation properties would lead to sound types showing shared features. This could be the case for the frequency properties of the 128 sound types identified across the floodplain channels. An important fraction of the sound types (77 % including the two most abundant categories: simple pulses and irregular sounds) were atonal and half of them covered a quite limited bandwidth between 2 and 9 kHz with a mean around 5.5 kHz. This shared frequency feature could constitute a variation of Morton's window defined for forest habitats (Morton, 1975): sound propagation in these water bodies might be more efficient in the 2-9 kHz frequency range. Such assumption still needs to be verified by conducting appropriate experiments that define the local sound propagation properties. Studying these acoustic properties is a real challenge in these heterogeneous and dynamic environments where sound propagation is far from simple and linear. The depth of the water body is the only factor that can be yet considered. Shallow water environments are known to act as a high-pass frequency filter whose cut-off frequency depends on water depth (Forrest et al., 1993). Here, the average depth of the channels was around 50 cm leading to a cut-off frequency of approximately 2.5 kHz in soft sediment and leaf litter bottom habitats according to the propagation model proposed by Forrest et al. (1993). This theoretical value fits well with the lower frequency limit of the 2-9 kHz bandwidth such that the structure of the environment might explain, at least partially, the main frequency feature of the sound recorded. If the AAH can explain shared properties, it can also be invoked to explain differences among communities if these communities evolved in distinct environments. Obvious differences in ground morphology, sediment nature, and vegetation occurred among the studied channels (Paillex et al., 2007; Riquier et al., 2015). These differences could have provided distinct transmission patterns and background noises that may have played a role in the distinctions between communities.

Acoustic competition within an assemblage of species might also foster diversity. This process is the core of the acoustic niche hypothesis (ANH) that stipulates that competition among soniferous species leads to a time and/or frequency partitioning of the acoustic space such that an over-dispersion of time and frequency features should be observed and therefore an important acoustic diversity should emerge (Krause, 1987). The ANH is the subject of ongoing debates because of the difficulty to experimentally test the hypothesis and due to diverging observations on the expected over-dispersion of acoustic features (see Schmidt et al. 2016 and Ruppé et al., 2015, for contrasted opinions). Although the potential effects of inter-specific acoustic competition on the community structure was not directly estimated here, the distinctiveness in the sound types observed within each community suggests that competition may have shaped acoustic diversity by an avoidance of signal overlap. However, such an assumption needs to be supported by behavioural and ecological studies defining the exact degree of overlap between signal traits and the recognition space of each species as defined by Amezquita et al. (2011). In addition, estimating the degree of “phylogenetic inertia” of the acoustic signals is also essential as divergence in signals could result from communities with wide taxonomic diversity, rather than competition for acoustic space (Goicoechea et al., 2010).

Lateral connectivity is a primordial environmental variable that testifies the past hydro-morphological processes undergone by the channels. Lateral connectivity, which combines effects of the frequency, duration and sheer stress during connections with the river has been identified as the major factor shaping the patterns of macro-invertebrate communities (Castella et al., 2015). A strong linear relationship was found here between composition of acoustic communities and lateral connectivity indicating that the composition of acoustic community progressively changes according to lateral connectivity. This striking result is in total agreement with what has been reported for macro-invertebrate communities (Castella et al., 2015). The linear relationship between community composition and connectivity was true, or at least stronger only if the site BEAR was removed from the analysis. The outlier position of this site conforms with its location in the floodplain, which makes it more influenced by a hillslope tributary, the Séran, than by the Rhône itself, both in terms of surface and groundwater supply. Riquier et al. (2015) also found BEAR to have peculiar sedimentological patterns, the site being "not yet adjusted to new conditions" induced by fluvial restoration. This singularity was also reflected in the macro-invertebrate community that was reported by Paillex et al. (2007) as being extremely dense and taxa-rich. BEAR also harbours taxa such as the mayfly *Siphlonurus sp.*, only found in BEAR, among 50 floodplain sites monitored along the French Rhône catchment (unpublished data from the Rhône restoration program). Therefore such an outlier position concords with previous observations, suggesting that an ecoacoustic approach can detect outliers.

The identification of the link between lateral connectivity and acoustic communities including the detection of an outlier in agreement with previous studies supports the idea that ecoacoustics can work as a valuable option to better understand the effects of connectivity and therefore the complex functioning of the floodplain ecosystem. The development of real-time monitoring tools is necessary to orient the practitioners' decisions in such threatened habitats. As already advocated (Desjonquères et al., 2015), the ecoacoustic investigation opens up new perspectives for the non-invasive and real-time monitoring not only of terrestrial and marine but also of freshwater environments.

3.2.5 Acknowledgements

We are indebted to Claude-Julie Parisot who unexpectedly initiated this project. We thank David McCrae and Hélène Siméant for collecting the connectivity data and helping with field work. We are grateful to Roger Mundry (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany) for providing R functions to study GLMM stability, to Peter Naylor for reviewing the English, and to Juan Sebastian Ulloa for his helpful insights on early drafts of the manuscript. This project was supported by a fieldwork grant from the LabEx BCDiv, an ENS PhD grant (CD), and a Post-doctoral grant from the Fondation Fyssen (DL).

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3.3 Additional results

Following the preparation of the manuscript reported in section 3.2, we conducted further analysis.

As detailed above, we found that acoustic community composition changed progressively with connectivity. Our study opens the question of how this pattern emerges. There are two main hypotheses to answer this question:

- Macro-invertebrates and fish communities which determine the acoustic community are influenced by connectivity.
- The physical characteristics of the sites, linked to lateral connectivity offer very different acoustic propagation conditions that are factors influencing the acoustic communities (AAH)

These two hypotheses are non mutually exclusive, and those two processes could act simultaneously. Here is a preliminary analysis to test each of these two hypotheses.

3.3.1 Between communities

3.3.1.1 Biological composition

We investigated the macro-invertebrates community composition of each of the site and tested this composition in relation to the lateral connectivity. We used a similar bCA multi-variate procedure.

Macro-invertebrates were collected in 2014, before and after the acoustic recordings. The sampling procedure for macro-invertebrates were fully detailed in Castella et al. (2015). The sampling consisted in 6 samples per site (3 collected in spring and 3 in summer) identified when possible to species level.

The bCA revealed distinct macro-invertebrate communities for each site and confirmed the specificity of the site BEAR, appearing relatively far from the other sites on the second axis of the bCA (Figure 3.9), as it was found for the acoustic community composition. The relationship between macro-invertebrate composition and lateral connectivity appears less clear than in Figure 3.7 where the sites appear clearly ordinated along connectivity. However plotting the second bCA axis with connectivity revealed the same trend as for acoustic communities: a potential linear relationship between the second bCA axis and lateral connectivity (Figure 3.10), which still need to be statistically tested with linear models.

This analysis therefore suggests that acoustic communities are strongly determined by macro-invertebrate communities. To confirm this result, the association between species and sounds is required.

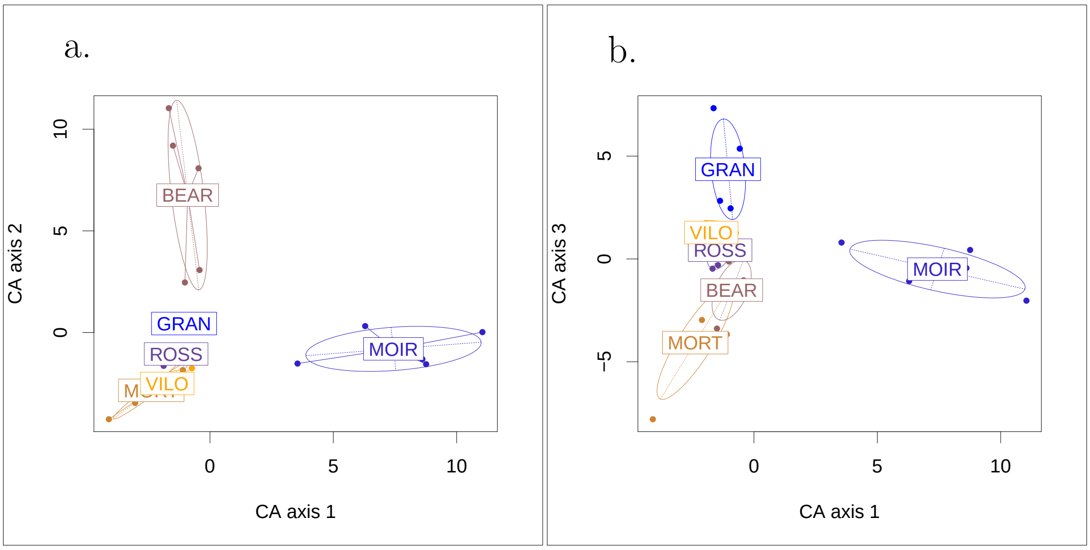

Figure 3.9 – **Between Correspondence Analysis (bCA) applied to the composition of macro-invertebrate communities**. The plots a. and b. are projections of the composition of the macro-invertebrate communities on the first three axes of the bCA. The sites were used as factors for variance maximization. Each point in the plot corresponds to the composition in macro-invertebrates of each macro-invertebrate community sample. The distance between points indicates composition dissimilarity. The dispersion ellipses surround the position of macro-invertebrate community providing an index of the dispersion around the centroid (67% of the macro-invertebrate compositions are expected to be in the associated ellipse). The names of the sites and their characteristics are detailed in section *Study sites*.

3.3.1.2 AAH

We then explored the average duration and dominant frequency of the sound types found in each site.

We found that acoustic characteristics of sound types found in each site were ordinated by connectivity except for the site ROSS (Figure 3.11). As stated before, lateral connectivity is an environmental variable summarising several physical and chemical variables in the secondary channels (sediment size diversity, sediment composition, water conductivity and vegetation cover). The physical variables of lateral connectivity may be strong determinants of sound propagation in freshwater environments. The link between connectivity and acoustic characteristics in the sites illustrated by the Figure 3.11 suggests that acoustic features may be influenced by the habitats as stated by the AAH. That is, sound production is adapted to the habitat

Lower connectivity sites such as VILO or MORT are highly vegetated with soft sediments bottoms whereas sites with high connectivity such as MOIR or GRAN are less vegetated and with more gravels and pebbles in the sediments. Here, we found sounds of higher frequency and shorter duration in vegetated environments. This appears surpris-

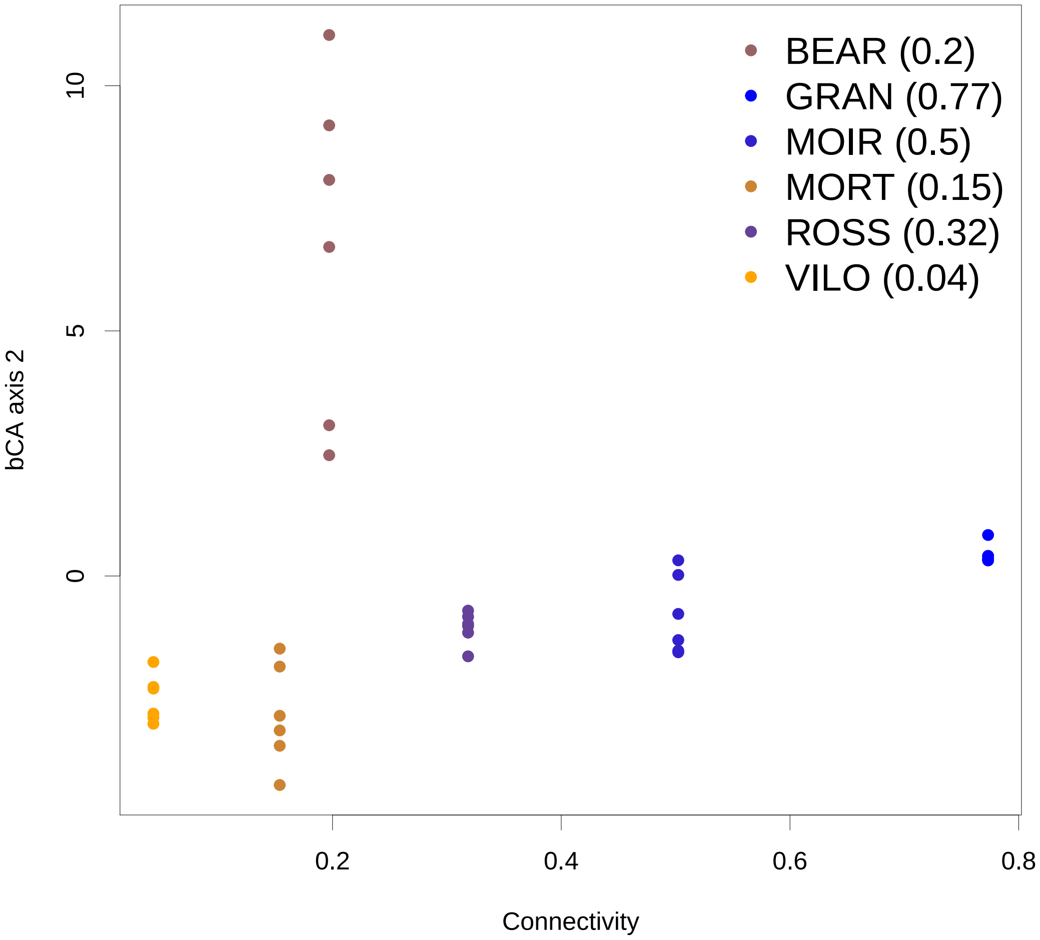

Figure 3.10 – **Relationship between lateral connectivity and the second bCA axis for the macro-invertebrate communities.**

ing when comparing with results found in birds where forest environments are associated to songs of lower frequency and of longer duration. Up to now, the differences in sound propagation between environments with different density of vegetation have not been investigated. It would be interesting to test the propagation patterns in these environments. For instance, according to our results we would expect ROSS to have similar propagation characteristics as VILO.

3.3.2 Within communities

Both the biological composition and the environmental conditions appear related to community composition and acoustic characteristics. How are acoustic characteristics distributed within each community ? We test here whether dominant frequency of the sound

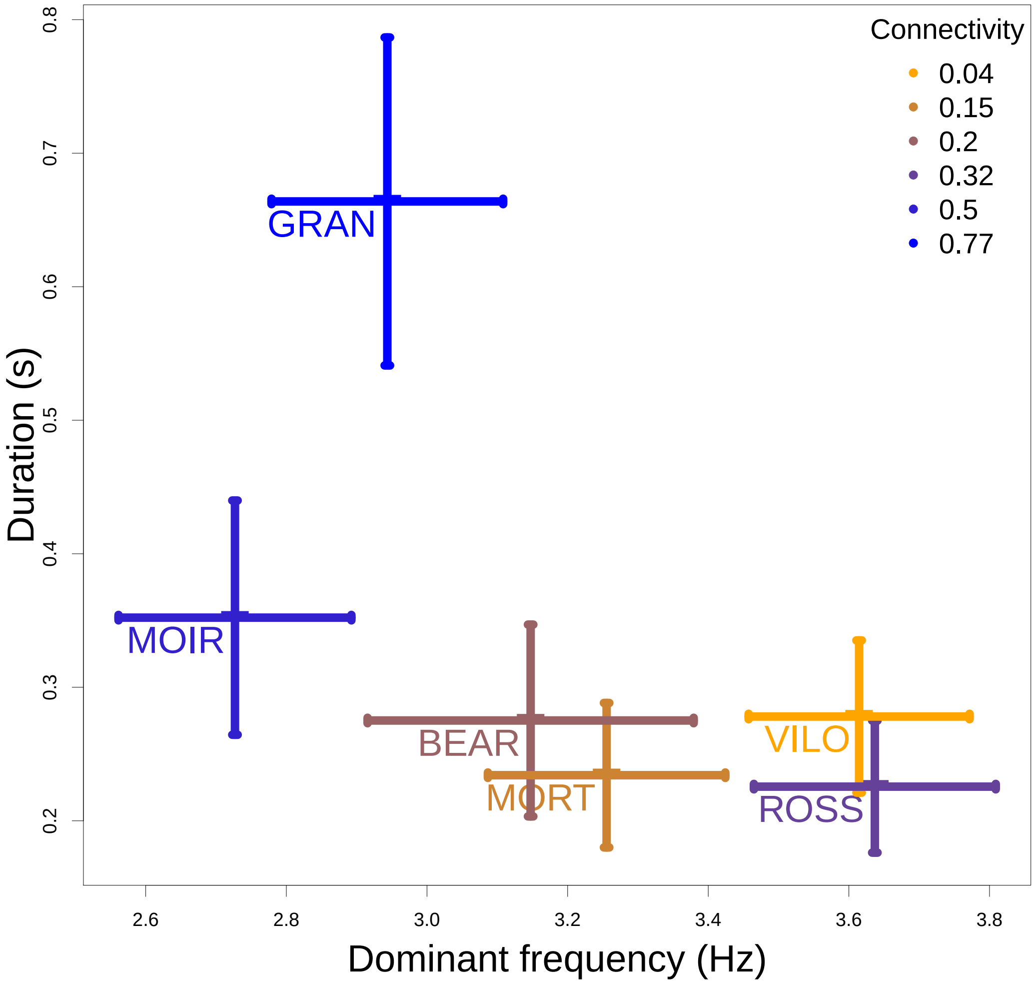

Figure 3.11 – **Average acoustic characteristics of sound types in each site**. The dispersion of sound dominant frequency (Hz) and duration (s) are shown with 95% confidence interval segments. The intersection of the segments corresponds to the mean. Each sites acoustic characteristics is based on the mean dominant frequency and duration of the sound types normalised by the number of recordings in which they occur.

types are clustered around a specific value, fully random or evenly space resulting in the three following predictions for acoustic distance between sounds within a community as stated in Tobias et al. (2014) (Figure 3.12):

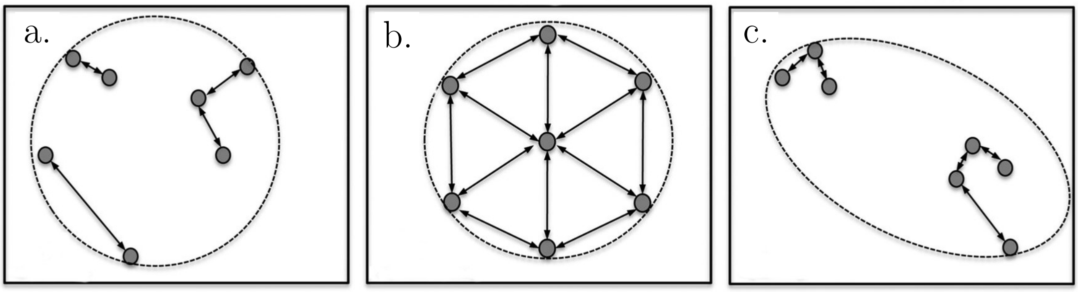

Figure 3.12 – **Three alternative hypothesis for acoustic characteristic spacing**. a. Random, b. Evenly spaced, c. Clustered (after Tobias et al., 2014)

- Random
- Evenly spaced (suggesting acoustic partitioning patterns, Acoustic Niche Hypothesis)
- Clustered (suggesting acoustic filtering patterns, Acoustic Adaptation Hypothesis)

To test how dominant frequencies are distributed in acoustic communities of the secondary channels, we compared observed communities to random communities thanks to a permutation test. We achieved this procedure for each site separately as they were shown to host distinct acoustic communities.

For each recording within a site, the difference between dominant frequencies of all sound types pair was computed. These differences were summed to evaluate the frequency distribution and its level of dispersion per recording. The median of these values were used as the test statistics.

To obtain random communities, we permuted the sound types within sites, resulting in the same number of recording with the same sound type richness per recording but different compositions. Then, the measures of frequency distribution were repeated to assess frequency dispersion of random communities.

Table 3.3 – **Results of the permutation tests comparing observed acoustic communities and random acoustic communities for each of the six sites**. The observed value corresponds to the median distance within the real observed community. The 5% statistic value is the value of the median distance for random communities corresponding to the 5% quantile.

Site	**Number of permutation**	**Observed value**	**5% statistic value**	**p-value**
BEAR	1000	50.170	46.121	0.004
GRAN	1000	76.662	41.5255	0
MOIR	1000	48.682	25.409	0
MORT	1000	73.388	51.142	0
ROSS	1000	89.204	67.8535	0
VILO	1000	86.999	85.6105	0.018

The observed communities in the six sites displayed an overdispersion of their frequencies, suggesting an evenly space pattern of frequencies. This suggests that a frequency partitioning (Acoustic Niche Hypothesis) may occur in these environments (Table 3.3).

3.3.3 Conclusion

Our results suggest that the biological and environmental conditions act simultaneously on acoustic communities composition and acoustic characteristics.

Within communities, the results of our permutation test showing an evenly spaced pattern of the dominant frequency suggests that an acoustic partitioning is acting to shape each acoustic community.

These preliminary tests could be completed by further experiments to test the relative importance of each of these processes and to control for confounding factors such as phylogenetic distances. Moreover, propagation tests could be conducted to test whether the different site have different acoustic propagation properties which could explain the observed differences in acoustic characteristics.

Chapter 4 :

Acoustics monitoring of the effects of anthropogenic noise on an aquatic insect population

Experimental set up in the study pond, Vidauban (Var, 83), June 2015

4.1 Summary

Context Processes structuring acoustic population in freshwater remain unexplored. Anthropogenic noise is a growing threat to various environments. The potential impacts of this threat on an aquatic insect acoustic population have never been assessed.

Problematics

- What is the dynamic of acoustic activity of an aquatic insect population ?
- Is there an effect of noise on the acoustic activity level and dynamic of an aquatic insect ?

Methods Acoustic monitoring of an aquatic insect (*Micronecta scholtzi*) in a Mediterranean freshwater pond:

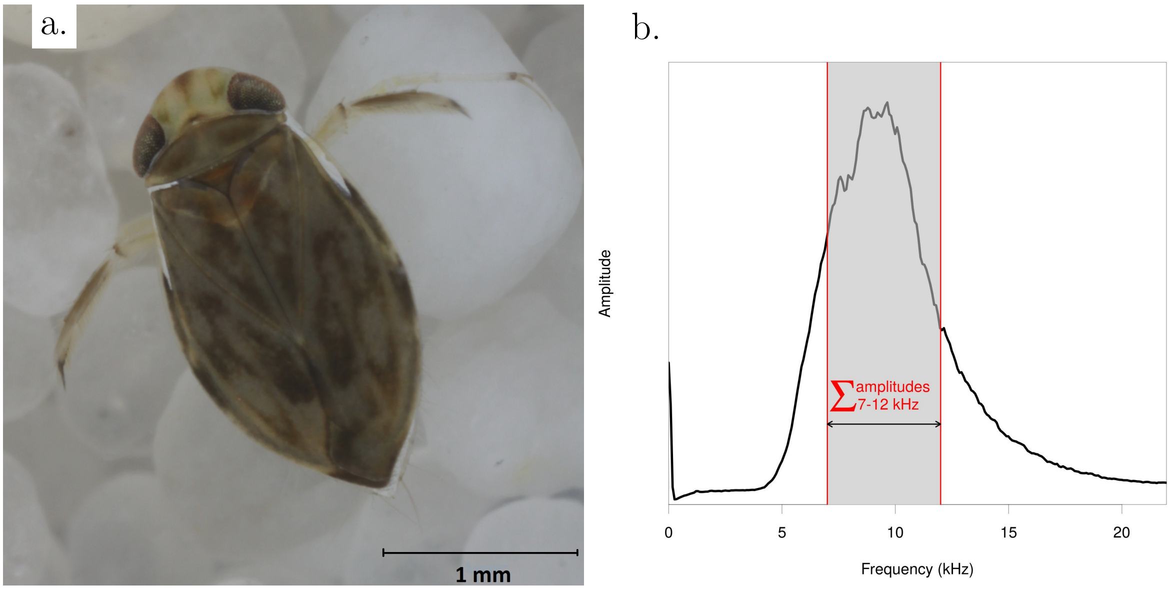

Figure 4.1 – ***Micronecta scholtzi* and the acoustic activity detection method**. a. *Micronecta scholtzi* (picture Jérôme Sueur), b. Spectrum of the sound produced by a population of *Micronecta scholtzi*. The detection method consists in measuring the amplitude in the frequency band of the stridulation of the species, *e.g* between 7 and 12 kHz

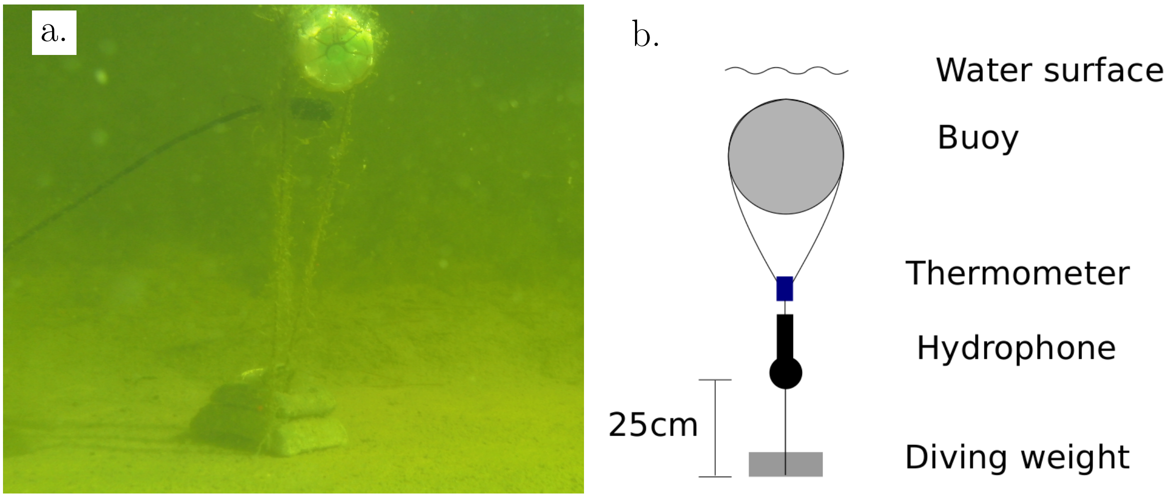

Figure 4.2 – **Underwater picture (a) and diagram (b) of the recording set up**. The recording set up includes a hydrophone and a thermometer attached to a rope tightened between a buoy and a diving weight.

- Noise played back between 3:00 and 5:00 am
- Functional linear models to test the effect of the noise on the acoustic activity of *Micronecta scholtzi*

Main results

- Efficiency of the method developed to measure the acoustic activity of *Micronecta scholtzi*
- Level of acoustic activity shows a negative relationship with vegetation and temperature
- Increase of the acoustic activity of the population in response to noise
- Delay in the acoustic activity correlated to the onset of noise
- Long term effects of noise: the acoustic activity level remained high and the timing delayed

Perspectives These results show for the first time effects of noise on an aquatic insect. These results suggest that anthropogenic noise can have long term consequences on aquatic insects.

Related communications and publications

- Acoustics monitoring of the effects of anthropogenic noise on an aquatic insect population **Camille Desjonquères**, Fanny Rybak, Juan Sebastian Ulloa, Alexandre Kempf, Avner Bar Hen, Jérôme Sueur. Manuscript in preparation for submission to *Global change biology.*

- Periodic acoustic activity of a water boatman (*Micronecta scholtzi*) in Mediterranean ponds. **Camille Desjonquères**, Fanny Rybak, Alexandre Kempf, Jérôme Sueur. 2014. Oral presentation at the Young Natural History scientists' Meeting, Muséum national d'Histoire naturelle, Paris.

- Monitoring the acoustic activity of an aquatic insect (*Micronecta scholtzi*) in Mediterranean ponds. **Camille Desjonquères**, Fanny Rybak, Alexandre Kempf, Jérôme Sueur. 2014. Oral presentation at the Ecoacoustics meeting, Muséum national d'Histoire naturelle, Paris.

4.2 Introduction

Current environmental deteriorations, including climate changes, habitat destruction, chemical pollution and noise stress, 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 et al., 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 observers (Hutto and Stutzman, 2009) and 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 reasonable solution to monitor populations of soniferous animals, such as birds, insects, amphibians and mammals, with a limited workforce and expertise (Laiolo, 2010; Sueur and Farina, 2015). Acoustic monitoring can be employed to assess the impacts of human-based disturbances such as climate changes on populations (Krause and Farina, 2016) or noise stress (Barber et al., 2011; Pieretti and Farina, 2013).

Noise pollution due to human activities has been identified as a growing threat for marine and terrestrial environments (Hildebrand, 2009; McGregor et al., 2013). The continuous noise related to transportation and industry and the impulse noise due to seismic air-guns are, among others, produced at so high pressure level that they can injure individuals (Popper et al., 2005; Popper and Hastings, 2009) or mask the signals used by animals for reproduction, maternal care, predation, environment sensing or orientation (Fletcher, 2007). Anthropogenic noise can also have an impact on the physiology, fitness and reproductive success of individuals (Rolland et al., 2012; McGregor et al., 2013) such that these individual damages can lead to significant modifications in population size, density and demography (Laiolo, 2010).

The extend of noise impact on natural populations has been mainly assessed in marine and terrestrial environments (Andrew et al., 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. For instance, Bolgan et al. (2016) estimated that the level of anthropogenic noise due to transportation in an Irish lake could reach 135 dB re 1 μPa at 15 m. Surrounding air-borne noises, such as noise due to road traffic or even recreational activities, can cross the air-water interface adding more anthropogenic noise to underwater environments (Kuehne et al., 2013; Holt and Johnston, 2015). Because sound travels faster and further in water than in air, high-level noise in freshwater environments may have a strong impact on animal populations as observed in marine environments (Tyack and Janik, 2013).

The potential effects of anthropogenic noise are usually assessed at the individual level by estimating either physiological or behavioural changes. Individuals show different strategies in response to noise exposure: they may try to escape noise by changing their location (McGregor et al., 2013), change their signalling rate (Blackwell et al., 2013), or adjust theirs signals in amplitude (Brumm, 2004; Parks et al., 2011), time (Foote et al., 2004; Fuller et al., 2007) or frequency (Roca et al., 2016). These behavioural adaptations have been mainly reported in birds, amphibians and mammals but very occasionally in insects although the diversity of their sounds often dominate the acoustic space (Stanley et al., 2016). Tree crickets of the genus *Oecanthus* have been proved to decrease instantaneously their signalling effort during periods of high levels of traffic noise (Costello and Symes, 2014; Orci et al., 2016) and the populations of *Chorthippus biguttulus* grasshopper have been shown to stridulate at a higher carrier frequency along roads than in quiet areas (Lampe et al., 2012). These behavioural 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 functioning (Francis et al., 2012), suggesting the occurrence of cascading impacts of noise.

We investigated for the first time the acoustic dynamics and the effects of noise on an aquatic insect population. *Micronecta scholtzi* (Insecta, Hemiptera, Corixidae) is a 2-2.5 mm water bug commonly found in temperate freshwater environments. The ecological importance of *M. scholtzi* has not been estimated yet but other species of *Micronecta* have been identified as good indicators of water quality in lakes (Jansson, 1977b). The male of *M. scholtzi* produces an intense stridulation (Sueur et al., 2011) that is likely to be involved in intra and inter-sexual relationships. Indeed in closely related species, male stridulation has been reported to induce positive female phonotaxis (King, 1999a), and to be produced in territory defence and aggregation contexts (Jansson, 1977b; Aiken, 1982b). The acoustic behaviour of *M. scholtzi* and other *Micronecta* species has only 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 sound choruses (Jansson, 1977b). This insect is therefore a good candidate for population acoustic monitoring. We developed a monitoring technique to continuously track in space and time the acoustic activity of a *M. scholtzi* population found in the south of France. This technique, easy to replicate, was then used to describe the acoustic dynamics of the population and to identify the environmental variables that could influence these dynamics. Finally, we tested the effect of an anthropogenic noise due to an engine on the intensity and timing of the population acoustic activity by an unprecedented playback experiment at the population level.

4.3 Materials and methods

4.3.1 Study site and data collection

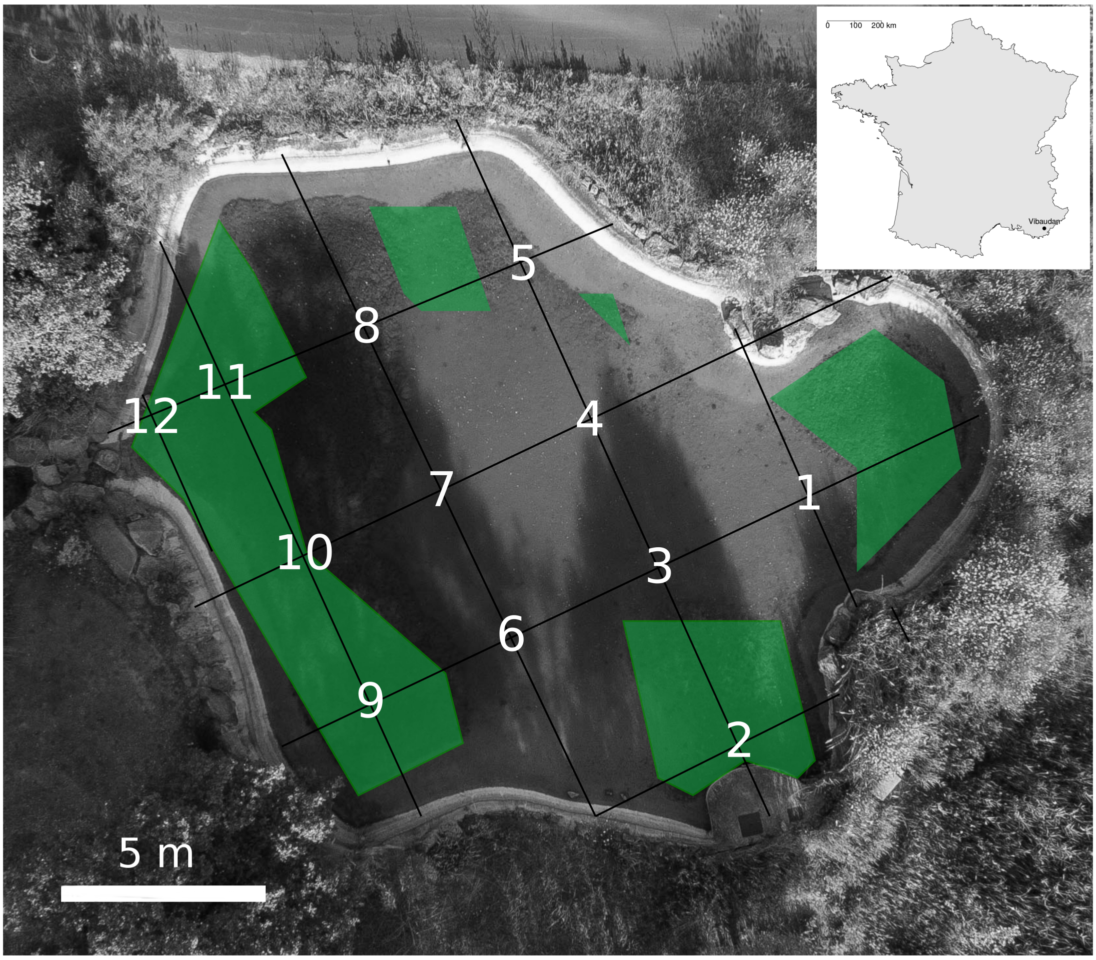

Figure 4.3 – **Schematic map showing the location of the recording stations in the pond**. The green shaded areas indicate the location of the vegetation and the numbers indicate the location of each recording station. The top left inset shows the general localisation of the pond in French mainland.

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, Figure 4.3). The pond had an approximate surface of 400 m^2 with a width ranging from 15 to 22 m (Figure 4.3). 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 algae species, *Chara globularis*. The spatial limits of the vegetation were visually assessed from the bank of the pond and from a small inflatable boat (Figure 4.3).

The pond was equipped with a network of twelve recording stations each separated by a distance to the nearest neighbour of 4.13 ± 0.88 m (mean ± sd, Figure 4.3). 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), a hydrophone (HTI-96, High Tech Inc., flat frequency response between 20 Hz and 40 kHz), and a thermometer (HOBO Pendant Temperature Data Logger, Onset, precision: 0.10°C). The recorder was hung on 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 thermometer was attached to the plastic bottle.

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

4.3.2 Study organism and its sound production

Micronecta scholtzi is a 2-2.5 mm water-bug (Insecta, Hemiptera, Corixidae) inhabiting running and stagnant freshwater habitats. The male produces an intense stridulation with a pulsed temporal structure lasting about 1 second and ranging from 5 to 22 kHz in frequency (Jansson, 1989; Sueur et al., 2011, Figure 4.4a). In the study pond, the main acoustic activity of *M. scholtzi* was estimated to occur between mid-June and mid-July (unpublished data).

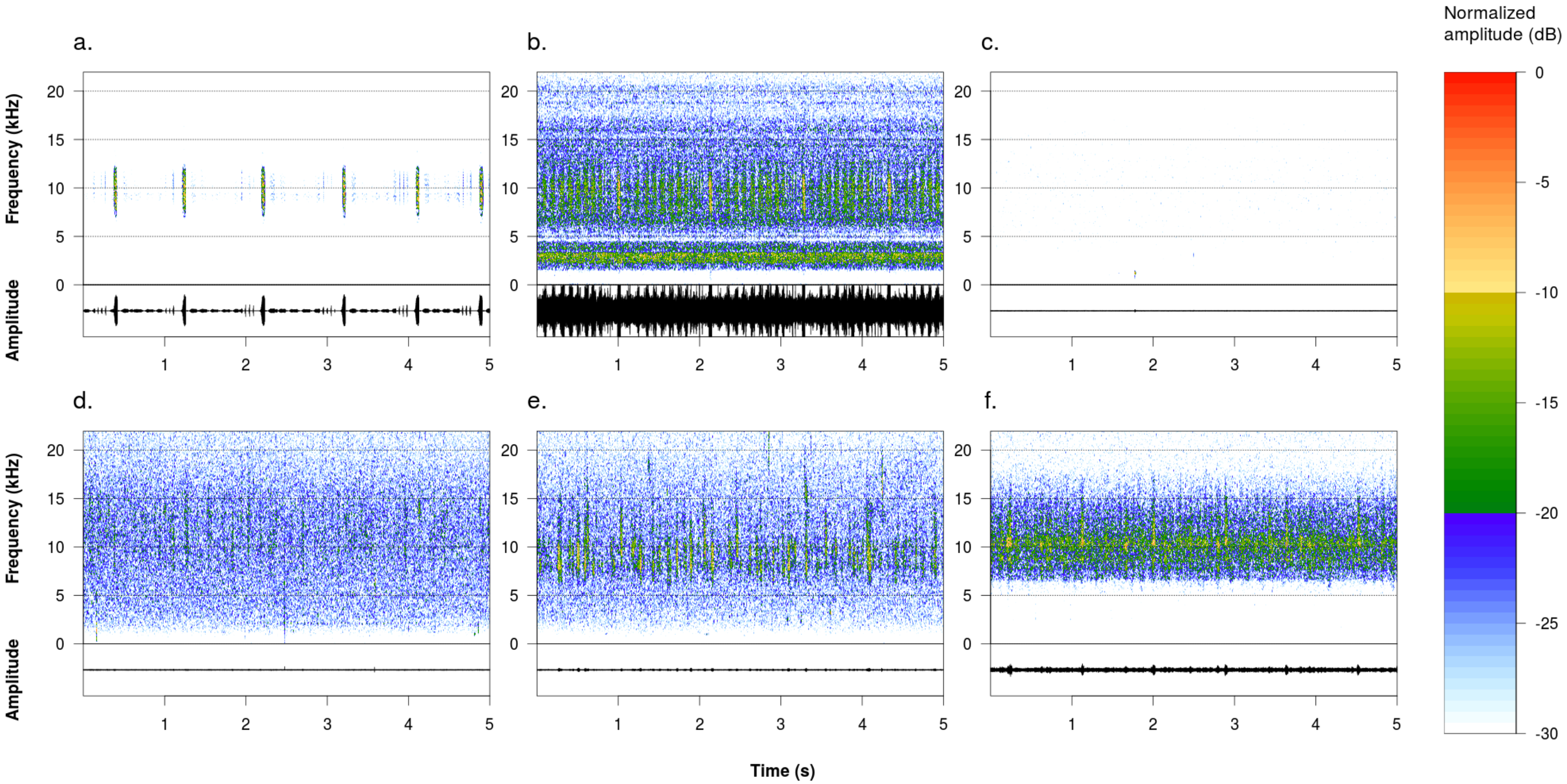

Figure 4.4 – **Spectrograms and oscillograms of the acoustic activity of *M. scholtzi*.** (a) recording of an isolated *M. scholtzi* male, modified from Sueur et al. (2011); (b) recording in the pond at the recording station 7 at 3:15 am on the 24th of June during the treatment phase when the engine was turned on; (c) recording station 12 at 4:00 pm on the 3rd of July with a listening score of 0: no activity, (d) recording station 12 at 4:00 pm on the 19th of June with a listening score of 1: distinct temporal pattern, with a low repetition rate (less than 13 repetitions in 10 seconds, corresponding to an estimation of one individual singing continuously, Sueur et al., 2011), (e) recording station 12 at 12:00 pm on the 19th of June with a listening score of 2: distinct temporal pattern, with a high repetition rate (more than 13 repetitions in 10 seconds, corresponding to more than one individual singing continuously), (f) recording station 12 at 12:00 am on the 27th of June with a listening score of 3: temporal pattern not identifiable (corresponding to a high density of individuals singing continuously). The recordings were not pre-processed, that is neither amplitude nor frequency filters were applied. Short-term Fourier transform parameters: 512 samples Fourier transform, 50% of window overlap, Hanning window, 30 dB dynamics amplitude

4.3.3 Automatic quantification of acoustic activity of *M. scholtzi* population

The simultaneous stridulations emanating from a population of *M. scholtzi* form a chorus in which the pulses of each individual song are not individually identifiable, but constitute a main frequency band from 7 to 12 kHz that can not be mistaken for any other type of sounds found in this environment (Figure 4.4d-f). A method was designed to automatically quantify the level of acoustic activity of *M. scholtzi* based on a measure of the amplitude found in the frequency band of *M. scholtzi.* The short-time Fourier transform was computed on the recordings 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 short-time Fourier transform 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, *e.g.* to obtain a mean frequency spectrum. Again, no scaling was applied at this stage so that the values of amplitude were raw values. The amplitude values of the mean spectrum for frequencies between 7 and 12 kHz were summed. This amplitude quantity, thereafter referred to as A_{7-12}, or $log_{10}(A_{7-12})$ when log-transformed, was computed with the seewave R package (Sueur et al., 2008). The validity of A_{7-12} to quantify the level of activity of *M. scholtzi* was tested through an aural assessment.

The aural assessment was achieved by investigating the first 10 seconds of a selection 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 during 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 hour 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 x 3 days x 6 times). The 02:00 am file was selected instead of the file recorded at 04:00 am because the peak of activity was observed at 5:00 am as reported in the section *Activity of the population in the absence of an anthropogenic noise*. A four level listening score was designed to assess *M. scholtzi*'s level of activity in each file: 0: no activity, 1: distinct temporal pattern, with a low repetition rate (Sueur et al., 2011, less than 13 repetitions in 10 seconds, corresponding to an estimation of one individual singing continuously), 2: distinct temporal pattern, with a high repetition rate (more than 13 repetitions in 10 seconds, corresponding to more than one individual singing continuously), 3: temporal pattern not identifiable (corresponding to a high density of individuals singing continuously) (Figure 4.4c-f). A Kruskal-Wallis test followed by pairwise Wilcoxon tests with a Bonferroni adjustment on the p-value were used to test whether the values of $log_{10}(A_{7-12})$ differed according to the listening score.

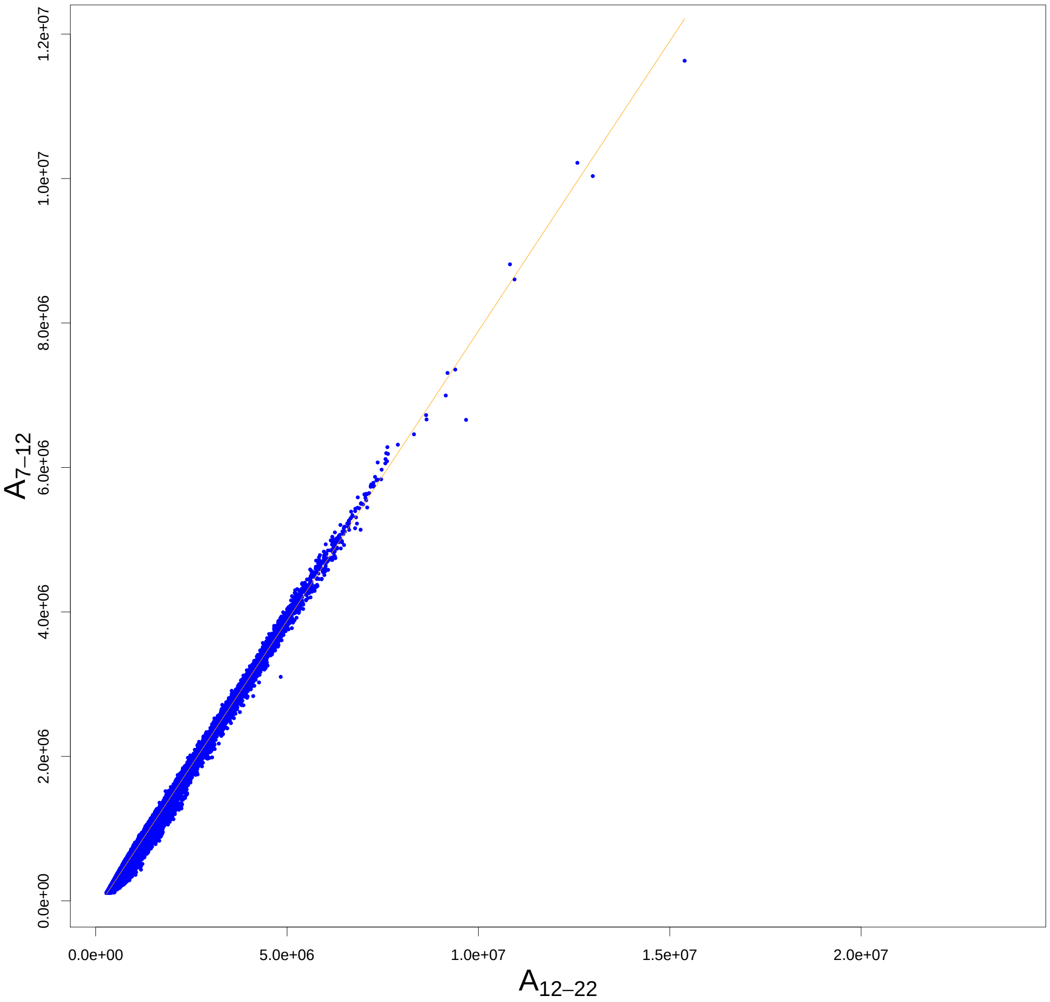

Figure 4.5 – **Relationship between A_{7-12} and A_{12-22}.** Each blue points corresponds to a recording. Yellow line shows the fitted linear model.

The level of background noise was quantified by measuring A_{12-22}, that is the summed amplitude values of the mean spectrum for frequencies between 12 and 22 kHz. The relationship between A_{7-12} and A_{12-22} was linear and positive (F-statistic: 5.3 106, adjusted R^2: 0.9956, d.f.: 1, 23498, p-value<0.001, Figure 4.5). Such a strong relationship indicated that the background noise was entirely determined by the level of activity of *M. scholtzi* confirming preliminary observations that suggested that *M. scholtzi* was the only sound source with frequencies above 7 kHz in the pond. Background noise was therefore not considered in further analyses.

4.3.4 Effects 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 $log_{10}(A_{7-12})$ measured during seven days, from the 16th to the 21st of June, in the absence of external noise disturbance. A major peak on the amplitude spectrum of the signal was found for a period of 24 hours.

The time (hour) of maximum acoustic activity of *M. scholtzi* was therefore assessed by looking within 24 hour windows. The time of maximum acoustic activity was measured as the median of all the time of maximum activity values obtained within the 24 hour windows for the seven 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 for the importance of the effects of temperature and vegetation (explanatory variables) on the acoustic activity level of *M. scholtzi* estimated with $log_{10}(A_{7-12})$ (response variable). The formula of the model was as follows:

$$y_{ij} = \mu + \alpha_i + \beta x_{ij} + \epsilon_{ij} \tag{4.1}$$

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

As the temperature and acoustic activity level were periodic time series, a Fourier basis, *e.g.* 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, *i.e.* the pairwise difference between the original signal ($log_{10}(A_{7-12})$ 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 $log_{10}(A_{7-12})$ or temperature. The presence or absence of aquatic vegetation at the hydrophone was encoded as a two level factor (Figure 4.3). Vegetation was modelled with a constant basis as vegetation did not change over the time of the study. Because functional linear models' theoretical null distribution of test statistics are not known, permutation tests were run to test model significance (Ramsay et al., 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 seven 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 (Ramsay et al., 2014).

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

The effect of environmental noise on the timing and amplitude of the acoustic activity of *M. scholtzi* population was tested using the engine noise of a local water pump. The engine of the pump produced a broadband noise with an irregular spectrum, covering *M. scholtzi*'s frequency band (Figure 4.4b). The pump engine could be turned on and off without running the pump such that only noise but no water flow was generated. The pump had never been turned on before the start of the experiment, making this noise an entirely new disturbance to this *M. scholtzi* population.

The response of *M. scholtzi* to noise was estimated on the long-term by conducting a three week experiment. 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 hour schedule described below (treatment). Finally, 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 such that it was not possible to disentangle the noise and signal sources. Therefore, the recordings achieved when the engine was turned on had to be excluded from the analysis. The engine noise had to be generated for a duration allowing a significant time of exposition to noise while enabling an extrapolation of missing values through a Holt-Winters filtering. It was therefore generated during two hours each 24 hour cycle, that is during 8% of the activity period of *M. scholtzi*. As the median time of maximum activity was estimated to occur at 5:00 am (see section *Activity of the population in the absence of an anthropogenic noise*), 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 of activity, right before the peak of activity when the population produced sounds but was receptive as it had not reached its maximum level.

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

The null hypothesis (H_0) was that the noise had no effect on the activity level, and the two alternative hypotheses were that the noise induced a decrease (H_1) or an increase (H_2) of activity.

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

$$y_{ijk} = \mu + \alpha_i + \beta x_{ijk} + \gamma_k + \epsilon_{ijk} \tag{4.2}$$

with y_{ijk} the $log_{10}(A_{7-12})$, i the index for the vegetation with a value of 1 for absence of vegetation and 2 for 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 pre-treatment, 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 were used to approximate temperature and $log_{10}(A_{7-12})$. The experimental phase factor, which consisted of three levels (pre-treatment, treatment, post-treatment) was added to the model as a dummy-coded variable with two piecewise constant functions. 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.

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

The null hypothesis (H_0) was that the noise had no effect on the timing of activity; the two alternative hypotheses were that the noise induced a time shift, either a delay (H_1) or an advance (H_2) in the timing of activity.

A cross-correlation procedure was used for each recording station to estimate the time shift between the three pairs of times series obtained coupling the pre-treatment, the treatment, and the post-treatment. The time series were scaled (null mean and standard deviation of one) over 24 hours. 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).

4.4 Results

4.4.1 Validation of the quantification of the level of acoustic activity

When considering all the 24,192 files, the amplitude measured between 7 and 12 kHz A_{7-12} had an average value of $1.02 \times 10^6 \pm 1.01 \times 10^6$ and ranged from 7.37×10^4 to 1.16×10^7. When considering the 216 files selected for the validation of the quantification of the level of acoustic activity, A_{7-12} fell into the same range of variation with an average value of $8.65 \times 10^5 \pm 9.33 \times 10^5$.

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

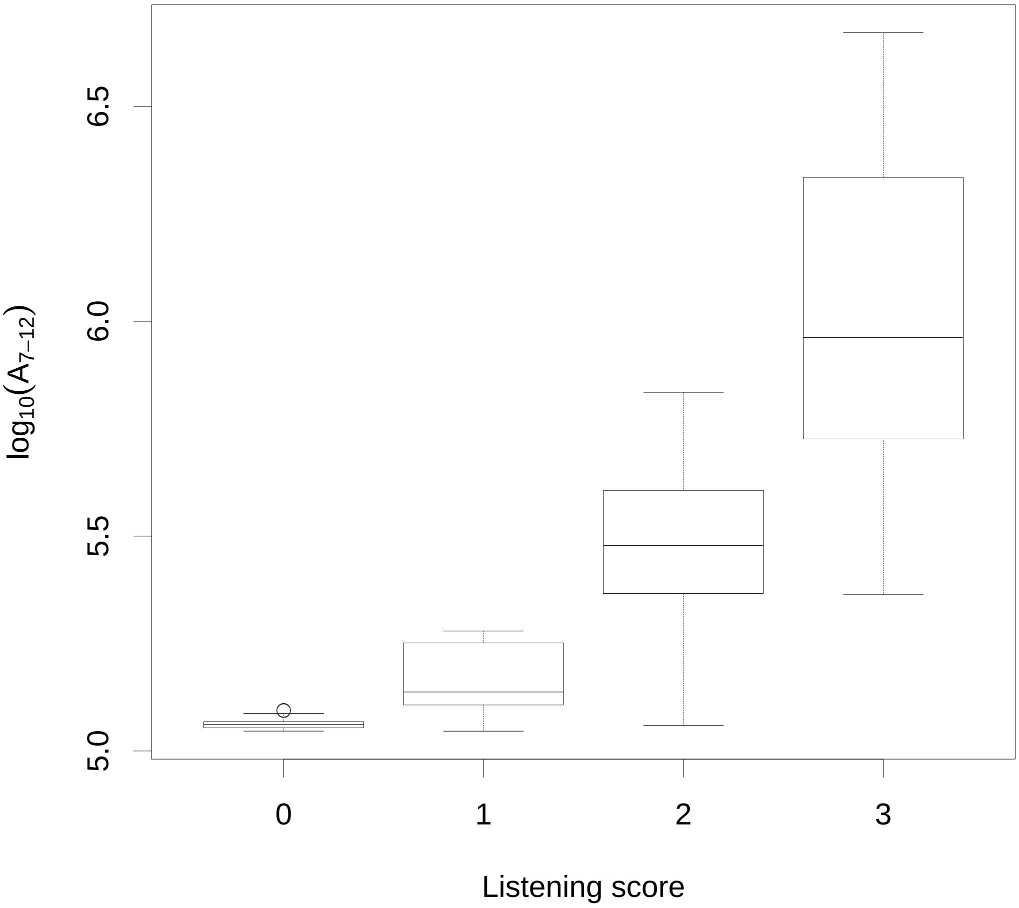

Figure 4.6 – **Relationship between the listening score and the acoustic activity** $log_{10}(A_{7-12})$. Boxplot showing the 25%, 50%, and 75% quartiles. The outliers measured as $Q_1 - 1.5 \times IQD$ and $Q_3 + 1.5 \times IQD$ are drawn as points (with Q_1 the first quartile, Q_3 the third quartile, 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 repetitions in 10 seconds, 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 repetitions in 10 seconds, corresponding to more than one individual singing continuously); 3: temporal pattern not identifiable (corresponding to a high density of individuals singing continuously). See Figure 4.4 for spectrograms and oscillograms illustrating the different levels.

The values of $log_{10}(A_{7-12})$ differed significantly between listening scores (Figure 4.6, Kruskal-Wallis test : χ^2=153.02, p-value<0.001, Pairwise Wilcoxon test with a Bonferroni correction : p-value<0.001 for all the pairs) and the $log_{10}(A_{7-12})$ increased with increasing listening score.

4.4.2 Activity of the population in the absence of an anthropogenic noise

The acoustic activity of *M. scholtzi* estimated with $log_{10}(A_{7-12})$ showed a regular 24 h 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 4.7).

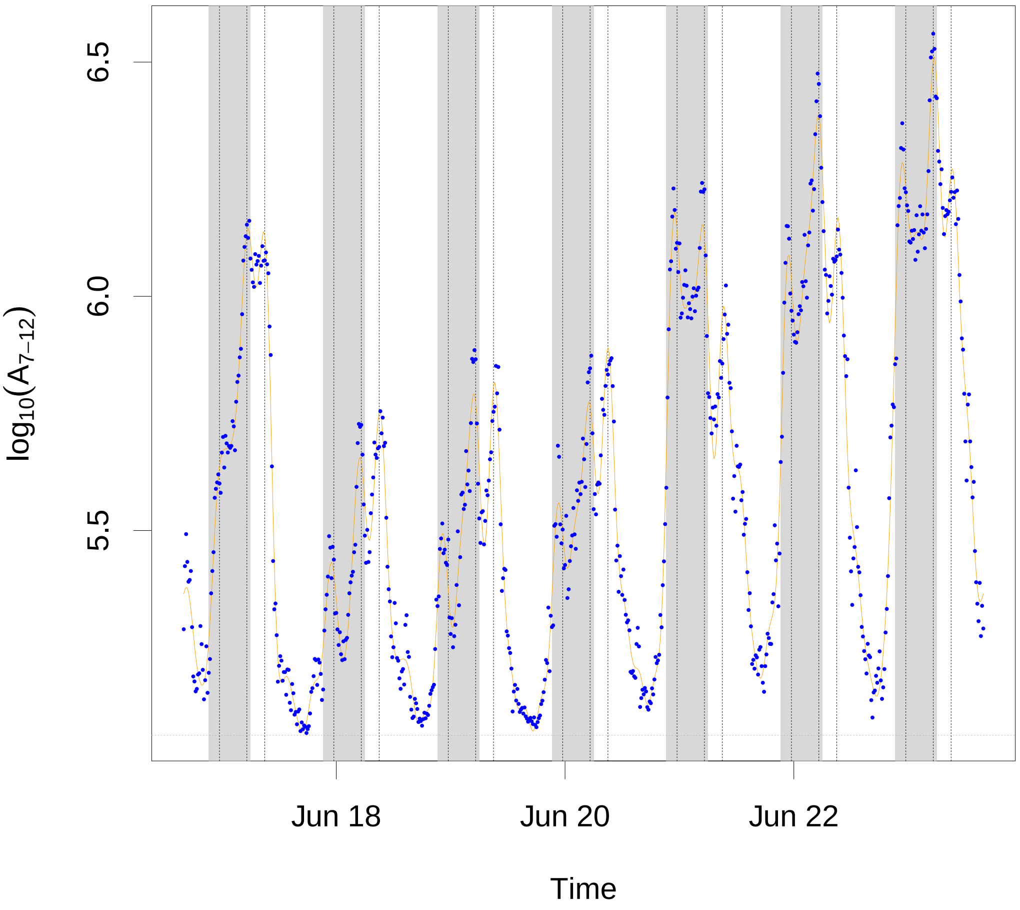

Figure 4.7 – **Time series of $log_{10}(A_{7-12})$ estimated at the recording station 12 for the first week (pre-treatment)**. The blue points are the values obtained for the recordings obtained every 15 minutes and the orange line is the functional model. The time is expressed as days; shaded areas highlight 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.

The functional linear model revealed a significant negative relationship between the acoustic activity $log_{10}(A_{7-12})$ and vegetation (permutation test: 1000 permutations, p-value<0.01, Figure 4.8a, Table 4.1). There was no significant relationship between daily temperature and the acoustic activity $log_{10}(A_{7-12})$ (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 $log_{10}(A_{7-12})$ and the instantaneous temperature (permutation test: 1000 permutations, p-value<0.001) with similar acoustic activity peaks at temperatures of 26.5°C and 28°C, corresponding to the temperatures recorded between 5:00-6:00 am and at 11:00 pm (Figure 4.8b, Table 4.1). Thus the instantaneous temperature influenced negatively the acoustic activity.

Table 4.1 – **Results of the functional linear model for the first week (pre-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 and the p-values are reported for each model term (p-value *<0.05, **<0.01, ***<0.001).

Term	**Estimate**	**CI$_{lower}$**	**CI$_{upper}$**	**Fstat**	**Degrees of freedom**	**p-value**
Intercept	5.75	5.66	5.83	(1)	(1)	(1)
Temperature(2)	-0.11	- 0.14	-0.08	0.35	1	0
Vegetation(3)	-0.17	-0.42	-0.06	(1)	(1)	(1)

(1) Not shown due to the lack of meaningful interpretation.

(2) Original mean and standard deviation of 27.93°C and 1.86°C respectively.

(3) Vegetation was dummy coded with absence of vegetation as the reference level.

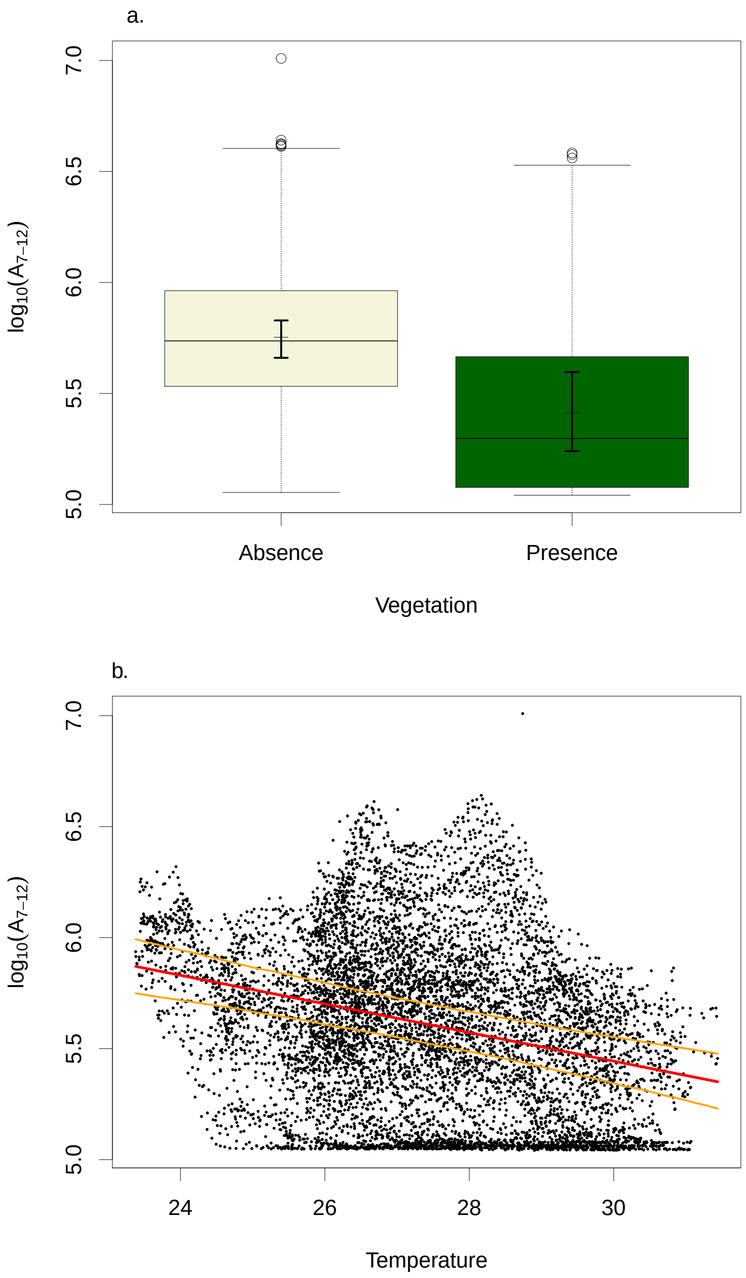

Figure 4.8 – **Relationship between $log_{10}(A_{7-12})$ and vegetation (a), and instantaneous temperature (b).** a. Boxplot of $log_{10}(A_{7-12})$ according to vegetation showing the 25%, 50%, and 75% quartiles and the outliers measured as $Q_1 - 1.5 \times IQD$ and $Q_3 + 1.5 \times IQD$ are drawn as points (with Q_1 the first quartile, Q_3 the third quartile, and IQD the interquartile distance). The bars in each box shows the estimation of the functional model and the 95% confidence interval for these estimations. b. Scatterplot of $log_{10}(A_{7-12})$ in function of instantaneous temperature. The red line shows the fitted functional linear model and the grey lines show the 95% confidence interval around the fitted values. The lower limit of $log_{10}(A_{7-12})$ values (5.03) corresponds to an absence of acoustic activity. The temperatures corresponding to peaks of acoustic activity are 26.5°C and 28°C.

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

4.4.3.1 Level of acoustic activity

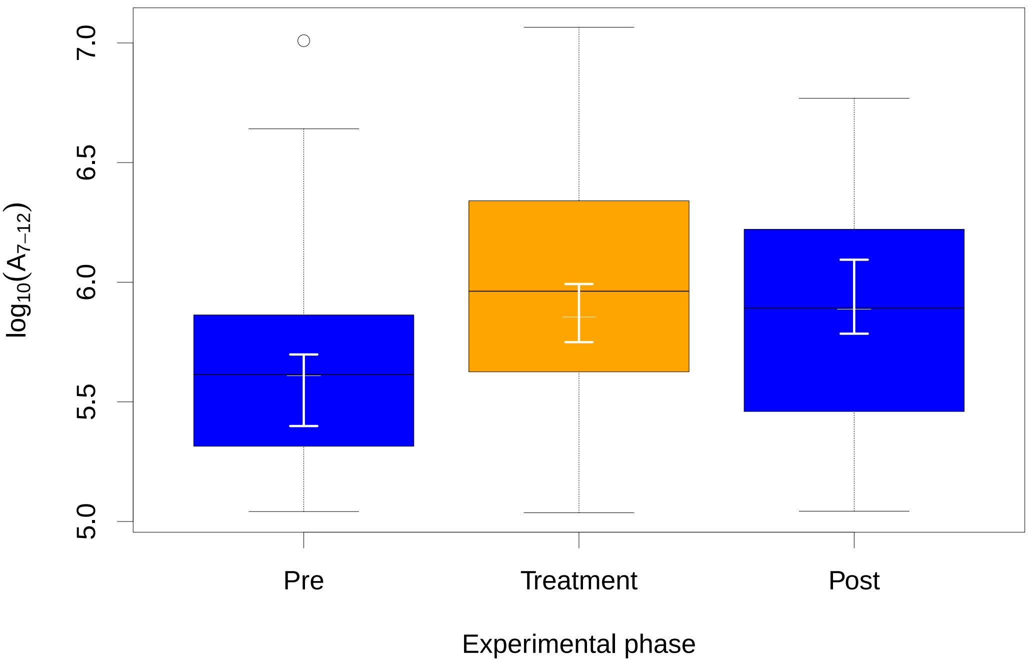

Figure 4.9 – **Relationship between $log_{10}(A_{7-12})$ and experimental phase**. Boxplot of $log_{10}(A_{7-12})$ according to vegetation showing the 25%, 50%, and 75% quartiles and the outliers measured as $Q_1 - 1.5 \times IQD$ and $Q_3 + 1.5 \times IQD$ are drawn as points (with Q_1 the first quartile, Q_3 the third quartile, and IQD the interquartile distance). The bars in each box shows the estimation of the functional model and the 95% confidence interval for these estimations.

The functional linear model showed estimations of the vegetation and temperature effects similar to the previous model only based on pre-treatment phase (Table 4.2), with a significant negative effect of vegetation (permutation test: 1000 permutations, p-value<0.01) and a negative effect of 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, p-value<0.01, Figure 4.9). Checking for pairwise differences, a near significant difference between the pre-treatment and treatment phases was found (permutation test: 1000 permutations, Bonferroni corrected p-value=0.06, Table 4.2). The treatment increased the level of activity of *M. scholtzi*. No other pairwise comparison showed a significant relationship (permutation test: 1000 permutations, Bonferroni correction, pre-treatment *vs* post-treatment: p-value=0.132, treatment *vs* post-treatment: p-value=1, Figure 4.9, Table 4.2).

Table 4.2 – **Results of the functional linear model over three weeks (pre-treatment, treatment, 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 and the p-values are reported for each model term (p-value *<0.05, **<0.01, ***<0.001).

Term	**Estimate**	**CI_{lower}**	**CI_{upper}**	**Fstat**	**Degrees of freedom**	**p-value**
Intercept	5.81	5.61	5.89	$^{(1)}$	$^{(1)}$	$^{(1)}$
Temperature$^{(2)}$	-0.15	- 0.26	-0.12	0.65	1	0.03
Vegetation$^{(3)}$	-0.48	-0.59	-0.35	$^{(1)}$	$^{(1)}$	$^{(1)}$
Treatment$^{(4)}$	0.25	0.18	0.43	$^{(1)}$	$^{(1)}$	$^{(1)}$
Post-treatment$^{(4)}$	0.28	0.18	0.58	$^{(1)}$	$^{(1)}$	$^{(1)}$

$^{(1)}$ Not shown due to the lack of meaningful interpretation.
$^{(2)}$ Original mean and standard deviation of 29.34°C and 2.21°C respectively.
$^{(3)}$ Vegetation was dummy coded with absence of vegetation as the reference level.
$^{(4)}$ Experimental phase was dummy coded with the pre-treatment phase as the reference level.

4.4.3.2 Timing of acoustic activity

The times series of $log_{10}(A_{7-12})$ showed a significant median positive delay of 10.6 minutes during the treatment phase (pre-treatment *vs* treatment, permutation test: 1000 permutations, p-value<0.01, Table 4.3) and of 13.9 minutes during post-treatment phase (pre-treatment *vs* post-treatment, permutation test: 1000 permutations, p-value<0.001, Table 4.3). No significant changes appeared between the treatment and post-treatment phases (treatment *vs* post-treatment, permutation test: 1000 permutations, p-value=0.168, Table 4.3).

The temperature was non-significantly delayed during the course of the experiment (Table 4.3). The sunset time shifts were negligible (Table 4.3). In contrast, sunrise times underwent delays which were not negligible and of the same order of magnitude as $log_{10}(A_{7-12})$ for the treatment *vs* post-treatment comparison (pre-treatment *vs* treatment: 2 minutes, treatment *vs* post-treatment: 3.4 minutes and pre-treatment *vs* post-treatment: 5.4 minutes, Table 4.3).

The delays for sunrise time were higher in the pre-treatment *vs* treatment comparison than in treatment *vs* post-treatment comparison (2 and 3.4 minutes respectively). If the delays of $log_{10}(A_{7-12})$ were solely due to the sunrise delay, the delay of pre-treatment *vs* treatment would be expected to be smaller than the delay of treatment *vs* post-treatment.

Table 4.3 – **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 pre-treatment phase). For sunrise and sunset, the mean, minimum and maximum values are reported. For the temperature and $log_{10}(A_{7-12})$, 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)**	**$log_{10}(A_{7-12})$ time shift (lower, upper CI, in minutes)**
Pre-treatment *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)
Pre-treatment *vs* post-treatment	5.4 (3, 8)	0.6 (0, 2)	0.4 (0, 1.0)	13.9 (8.2, 20.9) ***

However, the absence of significant delay for treatment *vs* post-treatment indicates that sunrise is not the only source of delay in $log_{10}(A_{7-12})$.

4.5 Discussion

Population monitoring is a crucial task for biodiversity assessment and conservation. Acoustic monitoring appears as a potential solution to follow populations of soniferous species. If several efforts are in development to acoustically track large animal species such as birds (Bardeli et al., 2010; Furnas and Callas, 2015), frogs (Garcia-Rutledge and Narins, 2001; Brandes et al., 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 et al., 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 appeared suited to follow the dynamics of acoustic activity of the waterbug *M. scholtzi*. The method had the great advantages to be uninvasive, time-effective and easy to replicate, three fundamental requirements for population monitoring studies (Blumstein et al., 2011).

Tracking in space and time the acoustic activity 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 the acoustic behaviour 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 et al., 2011). Second, the acoustic activity of *M. scholtzi* was characterised by a regular succession of three peaks at 5:15 am, 9:00 am and 11:30 pm. The dynamics of insect choruses have been shown to be mainly determined by competition between rivalling males. Each chorusing male would sing at least as much as its neighbours such that the information embedded in its signal is not masked (Greenfield, 2015). This competition induces an increase in calling activity that can be counterbalanced by the energy cost of signalling. Competition and energy expenditure might then explain alternation of calling and silence bouts (Greenfield, 2015). Here, the chorus temporal pattern could emerge from a combination of constraints related to diurnal rhythms (*e.g.* photoperiod, temperature), inter-individual competition, and signalling energy cost (Greenfield, 2015).

Acoustic monitoring could highlight a peculiar spatial organisation of the population with a negative relationship between acoustic activity and vegetation. The green algae 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, 1977a, ; personal observations). Interestingly, free sediments are associated to favourable conditions of sound propagation with less sound distortion due to obstacles. Such microhabitats would then be chosen also for acoustic properties of the environment allowing an efficient signal transmission, as stipulated by the acoustic habitat hypothesis (Marler and Slabbekoorn, 2004; Mullet, 2016).

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 of acoustic activity is expected to occur when the temperature augments towards an optimum (Begon et al., 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 of 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 reached 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 and Seura, 1986).

The engine noise used for the three-week playback experiment did not inhibit but increase the acoustic activity of the population of *M. scholtzi*. This was quite unexpected, as in other experiments testing the response to noise, insects decreased their calling behaviour when subjected to traffic noise (Costello and Symes, 2014). Here, the anthropogenic noise had an immediate stimulating effect on the population. At the individual level, the increase of the acoustic activity could be related to both an increase of stridulating rate, and/or an increase of the amplitude such that the message carried by the acoustic signal has a higher probability to overcome the noise. The Lombard effect corresponding to the increase of signal amplitude in response to the increase of ambient amplitude, has been well documented in terrestrial animals, in particular in birds (*e.g.* Zollinger and Brumm, 2011; Römer, 2013). Information redundancy, by increasing signalling rate in response to the increase of ambient amplitude has also been uncovered in several species (Bradbury and Vehrencamp, 1998; Brumm, 2006). The individuals forming the population of *M. scholtzi* might use such strategies by signalling louder to overcome the noise of the engine and/or more times to increase information redundancy. Another explanation could be related to acoustic competition among males and rough encoding-decoding processes. As mentioned above, insect choruses are now considered as a scene where males acoustically compete for accessing to females (Greenfield, 2015). Each male can be stimulated by surrounding males so that a chorus may emerge (Greenfield, 2015). This is probably the case of *Micronecta* species in which sound production not only attracts females but also maintains an acoustic territory in closely related species (Aiken, 1982a), is enrolled in male-male stimulation (Jansson, 1977a), and generates synchronised choruses (King, 1999c). Neural processes that encode and decode species-specific information in acoustic signals of competition between males can be rather simple in insects, as testified in cicadas, a group of terrestrial insects that belong to the same order Hemiptera as waterbugs. For instance, the chorusing behaviour of male of *Tibicina haematodes* can be elicited by a noise whose frequency band matches the frequency band of the male calling song (Sueur and 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. The individuals of *M. scholtzi* might consider the engine noise as a conspecific rivalling signal and therefore increase their sound production to maintain their territory and attract females. Only playback experiments conducted at the individual level could test these hypotheses.

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. This prolongation of their period of activity occurred always after the playback. This therefore induced a delay in the activity of the whole population.

These effects observed at the population level might be interpreted at the individual level by an excess of energy expenditure that might reduce individual fitness (McGregor et al., 2013). Moreover, the effects of noise during the treatment phase were also existing during the post treatment phase: neither the level nor the timing of the acoustic activity recovered the initial values of the pre-treatment phase during the post-treatment phase. As *M. scholtzi* was the most important element of the underwater soundscape, such prolonged effects of noise may not only affect 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; Solan et al., 2016; Simpson et al., 2016).

Ecoacoustics, through a three-week acoustic monitoring, proved to be a relevant approach to reveal patterns of activity of an aquatic insect. A rigorous and relatively simple protocol was sufficient to determine the spatial and temporal patterns of *M. scholtzi* acoustic activity and to identify effects of noise on this acoustic activity. These effects may be significant not only at the population level but also at the level of the community. 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 fishes.

4.6 Acknowledgements

We would like to thank the Fondation d'entreprise du golf de Vidauban, in particular Catherine Fournil for providing access to the study site and Pierre-Alexis Rault for his expertise on local fauna and flora. We thank Jean-Luc Pessel for his help during the field work. Our study would not have been possible without equipment kindly lent by Line Legall and Laurent Albenga. Finally, we thank Romain Garrouste and Laurent Lellouch for their help at the early start of the project. This work was supported by two fieldwork grants from the Labex BCDiv.

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Chapter 5 :

General discussion

Lône Bear (Savoie, 73), May 2015 (Picture: Diego Llusia)

The general aim of this PhD was to explore for the first time the acoustic diversity found in temperate freshwater by studying the patterns and structural processes of a selection of acoustic populations and communities.

This general discussion aims at summarising our current knowledge on the link between acoustic and the freshwater environments and at highlighting the areas where more research is required to better understand the processes structuring acoustic population, acoustic communities and soundscape. I hope to participate to the current development of ecoacoustics not only in freshwater environments but also in other environments.

5.1 Patterns: Acoustic diversity and dynamics in freshwater environments

There are three main levels of ecological organisation studied in ecoacoustics: acoustic population, acoustic communities and soundscapes which can be studied with different metrics (Figure 5.2). For acoustic population, those metrics are presence/absence, abundance and density. Acoustic communities can be evaluated by their diversity (richness and composition). Finally, for soundscapes, metrics consists in a characterisation of the relative importance of anthropophony, biophony and geophony and their spatio-temporal interplays. These three levels are composed of elementary sound components, I would suggest to qualify them as sound types. Each sound type can be defined by a set of parameters that I would suggest to name sound traits. The main sound traits of a sound type are its acoustic characteristics (*i.e.* frequency, duration, amplitude) and its temporal and spatial dynamics. Sound traits could be considered as an equivalent to the phenotypic traits of species in general ecology. Sound traits enable to position a sound type in the acoustic space (Figure 5.1). Therefore while sound traits consider the acoustic characteristics of sound types; acoustic populations, acoustic communities and soundscape are characterised by the presence of specific sound types at a given location and time.

5.1.1 Sound traits in freshwater environments

Sound traits are characterised mainly by five elements (Figure 5.1). The first three are acoustic characteristics of the sound: frequency, duration and amplitude. The last two are related to the place and time of emission.

Depending on the species and sound type, some sound traits such as timing of alarm calls, may be relatively plastic while other may be stable such as the sound frequency of many soniferous insects (Gerhardt and Huber, 2002) or the time their first song of birds taking part in the dawn chorus (Staicer et al., 1996). To identify the sound traits of a sound type, it is important to characterise inter- and intra-individual variability.

This implies to record several different identified individuals. For this purpose, either focal recordings in the field or laboratory recordings are necessary. Laboratory recording enables to control recording conditions which may impact the behaviour of the individuals or the conditions of transmission of the sounds (*e.g.* temperature, number of individuals). In our laboratory recordings, the aquarium and the level of water in it had a strong influence on the dominant frequency due to resonance effects (Chapitre 1, Akamatsu et al., 2002). Unfortunately, focal recordings in the field are not always possible, especially when targeting species which take part in choruses, or produce sounds in a noisy environment, or in turbid water.

The patterns of freshwater sound traits and in particular the acoustic features (*i.e.* frequency, duration and amplitude) were poorly described in the literature (Chapter 1). Frequencies range from 100 Hz for fishes to 100 kHz for Trichoptera. This frequency range appeared more restrained in the six secondary channels of the river Rhône (Chapter 3), where half of the sounds had their frequency between 2 and 9 kHz.

In terms of amplitude and duration, very little information is available. Some species produce short series of clicks (white-clawed crayfish, Chapter 1), and other very long sounds lasting several minutes (Ditiscid water beetle, Chapter 1). One freshwater insect holds the record for signal amplitude proportionally to size (Sueur et al., 2011), but in the laboratory and in the field, we recorded several sounds with low SNR suggesting a relatively low amplitude of emission for some of the sounds. This could be an issue to detect species using acoustic sensors (Chapter 1 and 2).

Temporal dynamics of activity of most animals are periodic, linked with diurnal or seasonal rhythm (Pijanowski et al., 2011). Most amphibians in France are acoustically active mostly in spring and during the night (Duguet and Melki, 2003). According to my personal observation with *Micronecta scholtzi* (Chapter 4) and the few papers based on field investigation (Jansson, 1974), it appears that most Corixidae species are more active during the night and mostly during spring and summer. To my knowledge, no information has been reported on the temporal dynamics of sound production for other groups such as fish, crustaceans and other insect species.

The ecological preferences and distribution of species are usually known, therefore spatial dynamics at the level of the watershed can be relatively easily to resolve. At a finer scale, however, spatial dynamics are relatively unknown. With the study on *Micronecta scholtzi* (Chapter 4), we revealed that over distances as small as 5 meters, the sounds recorded in a single pond could be significantly different, suggesting the existence of very fine scale spatial selection for sound production.

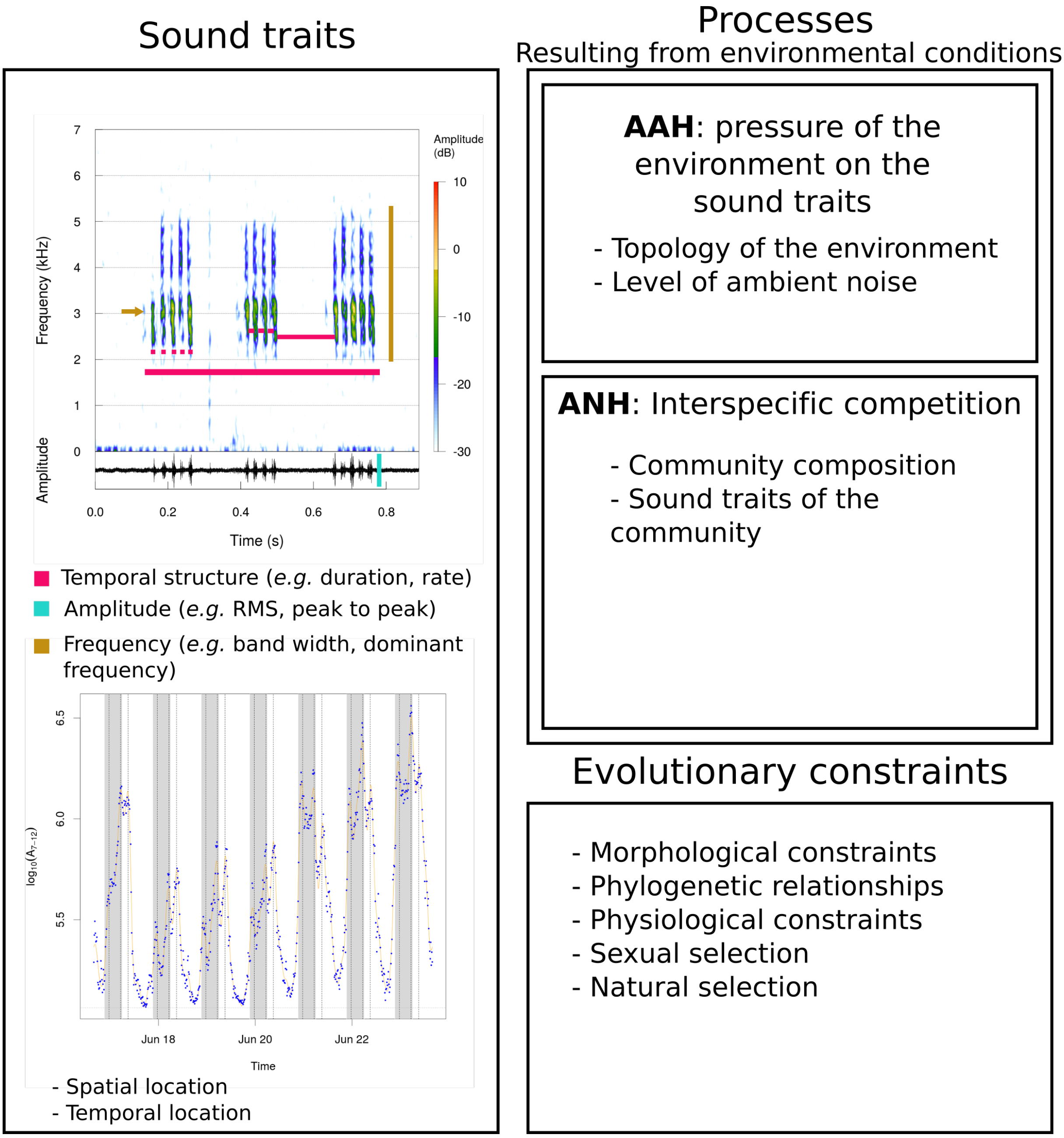

Figure 5.1 – **Summary diagram of evolutionary constraints and processes influencing sound traits**. The five main sound traits are shown along with processes that may influence those sound traits.

5.1.2 Acoustic populations in freshwater environments

Acoustic populations are acoustic assemblages constituted by one sound type probably due to one species. An acoustic population can be characterised by spatio-temporal dynamics of presence/absence, abundance or density (Figure 5.2).

In the field, several Passive Acoustic Monitoring (PAM) methods enable to estimate these metrics. The presence of a sound is usually relatively easy to determine as long as the traits of the sound are relatively well known. For abundance and density of sounds, counting sounds is not always straightforward (Kershenbaum et al., 2016). First, it is necessary to define what the sound unit is, which is relatively easy for a stereotyped sound but can be more difficult for sounds with variable duration (as in some cicadas) or variable motives (as in some birds). Moreover, for species producing sounds in a chorus, overlap may occur and prevent counting. Those factors have to be taken into account when designing a metric to measure acoustic abundance or density.

To study the presence of some species in freshwater, an acoustic approach may be more efficient than a classical sampling procedure. Indeed, some camouflaged or cryptic species have conspicuous acoustic productions. This is for example the case for Micronectinae which are usually small but produce regularly loud species specific sounds (Jansson, 1989; Sueur et al., 2011).

The patterns of abundance and density in freshwater environments appear relatively variable. Some sounds can be extremely abundant and dense, such as Corixidae sounds (Jansson, 1977, Chapter 4) while some other sounds are found only once in a while. For instance, in the six secondary channels of the Rhône (Chapter 3), 33 out of 128 sounds were singletons, that is were observed only once.

The temporal patterns of acoustic populations can be periodic as shown in the Mediterranean pond with *Micronecta scholtzi* (Chapter 4). The periodicity of the acoustic population was coherent within one pond, most activity occurring during the night. The same activity pattern was observed in two other ponds in the vicinity where the peak of activity occurred during the night (unpublished results of a preliminary study conducted in 2013) suggesting an intra-specific stability of temporal dynamics across sites.

Spatial patterns were also observed with this same species which appeared to prefer free sediment over vegetation (Figure 4.8, Chapter 4).

5.1.3 Acoustic communities in freshwater environments

The diversity at the acoustic community scale includes two components: richness and composition. Richness corresponds to the number of sounds in a community while composition focuses on the identity of the sounds and their relative frequency of occurrence.

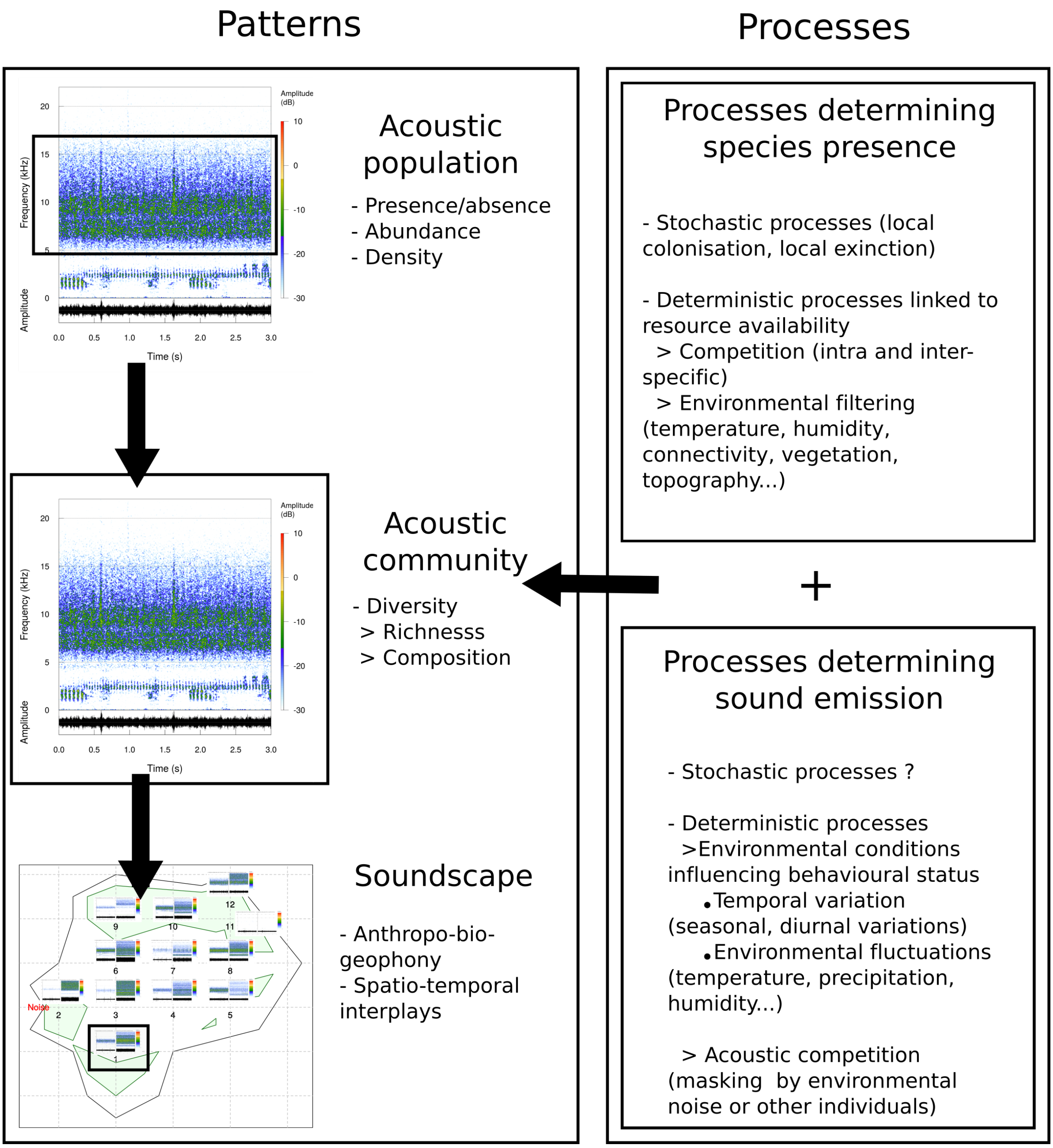

Figure 5.2 – **Summary diagram of patterns in acoustic population, acoustic communities, and soundscapes, and ecological processes associated to these patterns**. The three main levels of organisation studied in ecoacoustics are shown along with processes that may influence these assemblages.

Anderson et al. (2008) conducted a long term recording in two sites along the Hudson river and found a total of 62 sound types, allocated to fish sound production (4), biological sources (21), non-biological sources (5) and unknown sources (32). This represents a diversity of 44 sounds for the first site based on 104 hours of recording and 18 sound types for the second based on 60 hours of recording. While the authors identified different categories of sounds according to their potential emitters (fish, biological, or non-biological sources), I chose to avoid this type of classification of sounds. Indeed as no sound library of freshwater species is available such categorisations may be very speculative. Moreover, during oral presentations or discussions, I occasionally did blind tests with non-biologists as well as biologists and most people classify Corixidae songs as amphibian calls. A deeper knowledge on sounds is therefore required to be able to identify precisely the emitters and categorise them. This level of knowledge is not achieved yet.

Chapter 1, 2 and 3 revealed the important acoustic diversity found in freshwater environments. 271 species distributed in four taxonomic groups can be recorded in France. Because of both the low level of investigation on acoustics of freshwater environments and species pointed out in the review (Chapter 1), this number is likely to underestimate the actual diversity of emitters. Additionally, the number of sounds emitted by each species is unknown for most of the species, we can therefore expect an even greater diversity of sounds.

The average acoustic diversity per site was 24 in temperate ponds with a sampling effort of 120 recordings of 1 minute per pond (chapter 2) and 59 in secondary channels with a sampling effort of 240 recordings of 1 minute per site (Chapter 3). Such a diversity was unexpectedly high and had never been estimated in such environments before. Only two of the nine rarefaction curves obtained for the nine recording sites appeared to reach a clear plateau suggesting the existence of a significant amount of additional acoustic richness in seven out of nine sites.

Acoustic community diversity also revealed dynamics in space and time. Interestingly, in temperate ponds (Chapter 2), the periodicity of acoustic diversity of acoustic communities in three freshwater ponds was not in phase: each pond had a different time of highest activity. Knowing that those three acoustic communities were distinct, therefore probably composed of different species, this difference in timing may emerge from the fact that different freshwater species have different timing.

In the three ponds (Chapter 2) and 6 secondary channels (Chapter 3), we found that acoustic communities differed significantly between sites suggesting the existence of community specific acoustic signatures in freshwater environments. These acoustic signatures might be determined by different environmental conditions in these sites (Rossi et al., 2016).

5.1.4 Soundscapes in freshwater environments

Soundscapes are assessed by looking at the relative importance and spatio-temporal interplay of the three acoustic components of soundscapes: biophony, anthropophony and geophony.

Different methods can be used to study and compare different soundscapes including acoustic indices, semi-automatic detection and classification, or aural classification. In freshwater environments, I suggest to use mostly aural identification for two main reasons: i) in acoustic communities in temperate ponds (Chapter 2), we showed that most acoustic indices are more sensitive to SNR than to acoustic diversity, which could impair the interpretation of acoustic indices on acoustic communities but also on soundscapes, as they might be flawed to assess biophony, ii) the sounds found in freshwater environments had rarely been investigated before and for most of them there was no knowledge of their acoustic features (frequency and rhythm) and detecting automatically unknown sounds is, if not impossible, very tricky.

Therefore to use automatic identification in soundscapes, it is first necessary to identify clearly the different components (anthropo-bio-geophony) and their characteristics. Indeed, in freshwater, these characteristics are still under investigation. Although a first approximation has been used in Kuehne et al. (2013), considering that the energy between 1-2 kHz corresponds to anthropophony while between 2 and 8 kHz is biophony, a study on ferry noises showed that boat sounds could reach above this threshold and the main energy may be concentrated between 1 and 4 kHz (Bolgan et al., 2016).

Geophony linked to flow turbulence and sediment transport has been thoroughly investigated, in particular by Dr. Tonolla and his colleagues. They showed that the geophony could be detected through the investigation of nine frequency bands (Tonolla et al., 2010) and was highly dynamic in space and time (Tonolla et al., 2011).

Although general environmental conditions of freshwater environments is relatively straightforward to estimate roughly by general external aspect of the water and species composition (*e.g.* eutrophic *vs* heterotrophic), evaluating habitat condition according to soundscapes is difficult. For this PhD thesis, I have investigated several different freshwater environments in France (ponds in Saint-Pierre-lès-Némours (77), Quincampoix (76), Toulon (83), Bormes-les-Mimosas (83), lake in Vieure (03) and Saint-Bonnet-Tronçais (03), the river Loire in Blois (41), wetlands in Camargue (13)) and even in Germany, Denmark and Australia. These environments were investigated at different time of the day. The link between the habitat condition and sounds does not appear straightforward. This emphasises the difficulty to understand the general link between soundscapes and environments.

The patterns of acoustic diversity in freshwater environments appear to reveal some spatio-temporal structures and dynamics which are not fully random. These patterns are likely to result from the interplay of different ecological processes.

5.2 Processes: Potential assembly rules in freshwater environments

We first summarise here the theoretical processes acting in any environment (terrestrial, marine or freshwater) and then highlight the specificity of freshwater environments to infer the potential processes acting.

5.2.1 General processes

5.2.1.1 Determinants of sound traits in the environment

Sound traits may stem from a combination of several ecological and evolutionary factors (Figure 5.1).

Sound traits of a particular species are constrained by evolutionary factors such as morphological and physiological constraints. Closely related species are expected to be subjected to similar constraints due to their comparable morphology and physiology. Thus the potential effects of the environment on sound traits may be confounded with phylogenetic relationships. They therefore have to be accounted for in order to access the environmental effects.

Sound traits can also be influenced by the availability of the acoustic resource or acoustic space. Sounds in the environment are thought to have evolved towards an optimised transmission adapted to their function. Hence a double constraint is acting on the parameters encoding the information: these parameters should travel far enough without being degraded to be transmitted to the receptor and should avoid revealing the position of the sender to potential predators.

The Acoustic Adaptation Hypothesis (AAH) is linked to the filtering effect of the environment in which the sound is produced (Morton, 1975). Filtering occurs mainly due to the differential propagation and attenuation of sound frequencies in the environment. Environmental acoustic filtering is thought to result in a convergence of sound traits within environments. Environmental factors that can influence this effect are linked to the topology of the habitat and the level of ambient noise.

The Acoustic Niche Hypothesis (ANH) is linked to inter-specific acoustic competition (Krause, 1987). Acoustic competition occurs when two or more species are producing sound in the same acoustic space, leading to acoustic masking. The process of acoustic competition is thought to result in a partitioning of the acoustic space, each species occupying a specific non-overlapping acoustic niche in the environment. Factors influencing this process are the composition and sound traits of the acoustic community.

Another theory, conceptualised by Mullet (2016), states that species choose their habitats according to their sound traits and the acoustic transmission characteristics of the environment. This hypothesis called the Acoustic Habitat Hypothesis (AHH) along with the AAH tackles the issue of which pattern is the cause and which is the consequence: is the presence of a sound type in an environment due to the sound traits of this sound type or on the contrary are the sound traits the result of the pressure of the environment on the sound traits of a sound type ? In any case, this hypothesis suggests strong links and interactions between sound type presence and sound traits in the environment. We now focus on the processes determining sound type presence in the environment.

5.2.1.2 Determinants of the presence of a sound type in the environment

There are two necessary conditions for a sound to be found in a specific environment: i) the presence of the emitter and ii) the emission by the emitter (Figure 5.2). What are the factors and processes influencing these two conditions ?

The presence of an emitter in a specific environment is determined by general ecological processes which can be stochastic and/or deterministic (Chase and Myers, 2011). Stochastic processes include local random extinction or colonisation events. Deterministic processes are related to resource availability which is influenced by competition and environmental conditions such as temperature, vegetation or humidity and AHH.

The emission of sounds by emitters is determined by acoustic processes which may also be stochastic or deterministic. Stochastic processes have never been investigated or theorised before but may be linked to random calling patterns as those found in choruses (Greenfield, 2015). Deterministic processes are linked to acoustic resource availability influenced by competition (with other species, geophony and/or anthropophony) and environmental filtering which determine the species behavioural status such as temperature or humidity. Behavioural status is also influenced by temporal rhythms (diurnal and seasonal).

Indeed, these two elementary conditions, presence of the emitter and emission are confounded when looking solely at the sound emanating from an environment. The effects of general ecological and acoustic processes at stake may be disentangled by assessing the actual species richness, composition or density. This would therefore enable to dismiss the general ecological processes and focus on acoustic processes which have benefited from less investigation so far.

The two summary diagrams (Figure 5.1 and 5.2) show the ways in which ecological processes may influence specific sound traits or sound types in the environment. These processes act simultaneously and in interaction. Each of them influences sound traits and sound occurrence more or less strongly depending on the environmental conditions. Thanks to this first exploration of the link between sound and the environment in freshwater, we can start to ask : What are the specificity of freshwater environmental conditions ? What are the potential processes specifically acting in freshwater environments ?

5.2.2 Processes in freshwater environments

5.2.2.1 Determinants sound traits in freshwater environments

AAH The acoustic space of freshwater species differs from the ones observed in other environments (marine and terrestrial) for three main reasons: i) propagation medium (water), ii) physical habitats of freshwater environments (*i.e.* very variable topography, possibly very shallow and with various types of sediments), and iii) ambient and anthropogenic noise.

The physical habitat and propagation medium of freshwater environments mainly influence the propagation of sounds in water. The propagation of sounds in shallow waters is a complex phenomenon due to the multiple reflection and refraction by the water surface. Aiken (1982a) have shown that in lakes with gravels sediment bottom, sounds with a frequency around 5.5 kHz propagate the most efficiently. Moreover, Forrest et al. (1993), showed the existence of a high pass filtering effect of shallow water. The cut off frequency of this filter decreases with depth. In secondary channels of the river Rhône (Chapter 3), we measured frequency and duration of freshwater sounds. Half of the sounds were found between 2 and 9 kHz. This observed pattern may be the result of environmental filtering as suggested by Forrest et al. (1993) who state that shallow water applies a high pass filter with a cut-off frequency around 2 kHz for such a depth.

Although these two studies point out the importance of depth and topography, the effects of other factors of the physical habitat such as temperature, vegetation or sediment nature have not been evaluated in freshwater environments. In terrestrial environments, vegetation and temperature (*e.g.* Darras et al., 2016) as well as salinity and temperature in marine environments (Buckingham, 1992) have been demonstrated to influence strongly the propagation of sounds in the environment. Estimating their effect in freshwater environments would enable to estimate the extend of acoustic space available for sounds in freshwater environments. There are three main potential reasons explaining this lack of study on the propagation patterns in freshwater environments: i) freshwater environments have benefited from a relatively small number of studies so far, ii) freshwater environments are complex and dynamic (Ciruna and Braun, 2005), therefore finding standard set ups to test the effects of physical habitat in freshwater environments may not be so easy, iii) acoustic propagation in shallow water is relatively complex to model (see below).

Another important feature of inhabitants of freshwater environments as fish and invertebrates is their ability to perceive particle velocity instead of pressure (Nedelec et al., 2016). As stated in the *General introduction*, the relationship between pressure (p) and particle velocity (v) when the wave front can be considered as plane follows:

$v = \frac{p}{z}$, with z the impedance of the propagation medium.

In shallow environments such as freshwater environments, the wave front is not plane (Nedelec et al., 2016), thus this relationship is not true. This implies that the sounds recorded in the environment with a hydrophone might be quite different from what individuals perceive. Although we do not know how strong the decorrelation between particle velocity and pressure is, recording particle velocity would probably be more relevant to the investigated species.

As pointed out in the introduction of the chapter on *Micronecta scholtzi* (Chapter 4), anthropogenic noise is also present in freshwater environments. Indeed, terrestrial anthropogenic noise can be transmitted underwater (Kuehne et al., 2013). Moreover, recreational activities, transports and industrial activities appear to generate important sources of noise in freshwater (Bolgan et al., 2016).

ANH As stated above, the knowledge on composition and sound traits of acoustic communities is still relatively low.

The density of occupation by soniferous species could not be estimated precisely in this PhD, nevertheless, the average diversity per recording could be estimated. An average of 1.3 for the three ponds (Chapter 2) and 5.7 sound types on average for the six secondary channels (Chapter 3). This reveals a potentially low level of co-occurrence especially in the three ponds which may result in low acoustic competition. On the other hand, some recordings in the Mediterranean pond studied for *Micronecta scholtzi* (Chapter 4) showed patterns of co-occurrence which could indicate some frequency acoustic niche partitioning (Figure 5.3). This has to be statistically tested, to reveal how consistent this pattern is.

To test statistically the ANH, an analysis of the pattern of sound type co-occurrence in relation to the sound traits would enable to reveal whether similar sounds co-occur more, less or as much as expected by chance, as tested in the secondary channels (Chapter 3). Less co-occurrence than expected by chance would suggest the existence of an avoidance or partitioning. Such study requires to be undertaken at the community level, controlling for phylogenetic relationship between species. Such a statistical procedure to test this hypothesis has been undertaken for example in bird communities (Tobias et al., 2014) and cricket communities (Schmidt et al., 2016). One limit to these two studies is the fact that they are not able to consider the entire acoustic communities while it has been demonstrated that acoustic competition can also occur between phylogenetically distant species such as insects and birds (Stanley et al., 2016). In distantly related species, controlling for phylogenetic relationships may not be as critical as for closely related species. In secondary channels of the Rhône (Chapter 3), we could not access species identities, and thus could not control for phylogenetic relationship. With a permutation test, we revealed that co-occurring sound type frequencies where more divergent than expected by chance suggesting an acoustic partitioning.

Evolutionary constraints Evolutionary factors influencing sound traits in freshwater environments are still difficult to assess as until now we are not able to associate sounds and species. Moreover these constraints are highly linked to the two preceding hypothesis, for example, natural and sexual selection drive the evolution of sound traits due to the AAH and ANH.

5.2.2.2 Determinants sound type presence in freshwater environments

Processes influencing the presence of the emitter In freshwater, stochastic processes such as local migration are mainly linked to connectivity (Ciruna and Braun, 2005): freshwater environments can be seen as islands of water in a sea of land.

In freshwater, deterministic processes imply strong links with specific environmental gradients such as lateral connectivity in the case of the secondary channels in the Rhône floodplain channels (Castella et al., 2015; Lamouroux and Olivier, 2015).

Processes influencing the emission In freshwater environments, some environmental conditions such as temperature are more stable in water than air, however there are potential seasonal extreme events such as floods or droughts which may influence behaviour of local species. For example, *Eretes sticticus* has been observed to emit a specific sound in mass migration due to the desiccation of their pond (Kingsley, 1985).

As stated in section *Determinants sound traits in freshwater environments*, density of sound types may influence the intensity of acoustic competition which may then only take place significantly in environments with high levels of co-occurrence.

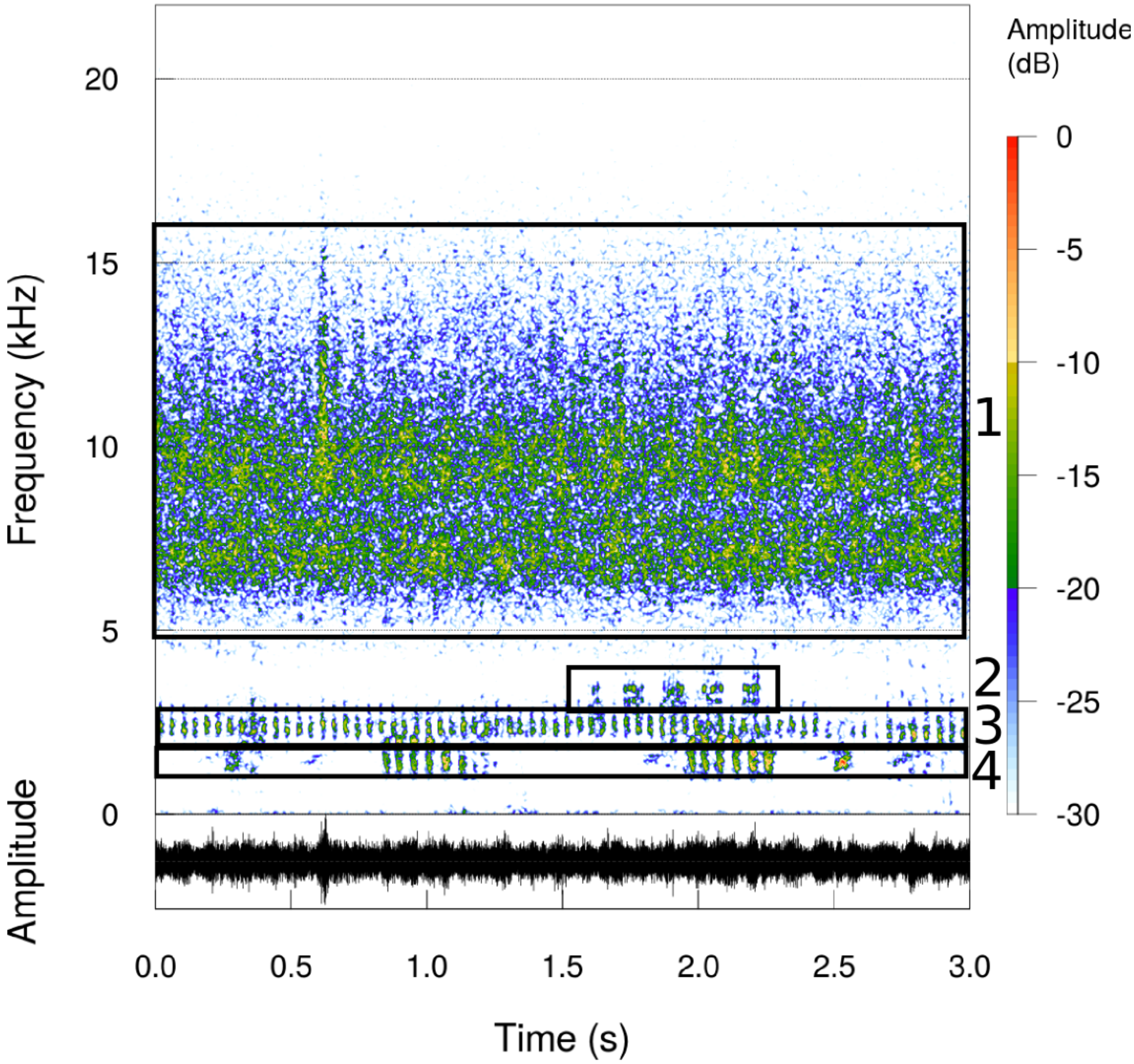

Figure 5.3 – **Spectrogram and oscillogram of a recording in the *Micronecta* study pond (Chapter 4) showing a potential frequency partitioning pattern of insect and frog species**. 1 - *Micronecta scholtzi*, 2 - *Sigara* sp., 3 - *Corixa punctata*, 4 - *Pelophylax ridibundus*

In the secondary channels of the river Rhône, as suggested in the section *Additional results* (Chapter 3), several processes may be interacting to result in the observed patterns. In this chapter, we demonstrated a link between acoustic community composition and lateral connectivity in riverine floodplain. Fish and macro-invertebrate community composition are also mainly determined by lateral connectivity in those environments (Castella et al., 2015; Lamouroux and Olivier, 2015, Figure 3.10). This pattern therefore probably stem from general ecological processes which influence the presence of specific emitters. We then investigated the sound traits of sound types found in each site, we found a potential link between connectivity and sound type frequency and duration (Figure 3.11). This link could either stem from general ecological processes or environmental filtering acting at the site level. Finally, when investigating sound traits inside each site, we found that dominant frequency of sound types in the acoustic communities were more divergent than expected by chance. This suggests a potential partitioning of the acoustic space within the six acoustic communities. To test actual processes in freshwater environments would require to know sounds, sound traits and phylogenetic relationship between species of the community similarly to what Schmidt et al. (2016) or (Tobias et al., 2014) achieved. Moreover, to confirm the potential acoustic filtering process requires assessing the sound propagation and ambient noise in the six sites. It could also be interesting to increase the number of investigated sites to have more robust and reliable conclusions.

Although the processes leading to the observed patterns are still very poorly understood in freshwater as well as in other environments, the growing knowledge on acoustic populations, communities and soundscapes opens the perspective of a derived application: Ecoacoustic Monitoring.

5.3 Perspectives in applied ecology : Ecoacoustic Monitoring of freshwater environments

As demonstrated in this thesis, several species and ecological levels of organisation (acoustic population, acoustic community and soundscape) produce sounds that may have an acoustic signature underwater. This property could be used to monitor freshwater environments. We discuss here what these soniferous components are, how we could integrate them for an ecoacoustic monitoring and finally we underline areas for further research to standardise this method in freshwater environments.

5.3.1 Population monitoring

As shown in the review (Chapter 1), at least 4 animal groups produce detectable sounds underwater. Those detectable sounds appear to be mostly species specific.

These sounds can be used to monitor populations in various contexts. As demonstrated with *Micronecta scholtzi* (Chapter 4), acoustic monitoring can be used to follow the response of a population to environmental changes. Other applications can be envisioned such as monitoring the presence of invasive (Tennessen et al., 2016) or threatened species (Dutilleux and Curé, 2016).

This type of application appears specifically adapted for freshwater environments for two main reasons. Firstly, the methods currently used to monitor populations consist mainly in netting and electro-fishing two invasive methods which can impact the health of captured individuals. Secondly, these methods do not allow a continuous monitoring. I therefore think that acoustic population monitoring should be developed further in freshwater environments.

Although our monitoring technique detected efficiently the acoustic activity of *Micronecta scholtzi*, this technique is unlikely to work well with species emitting sounds very rarely or quietly. Indeed for any detection method, the SNR may be an issue. Therefore automatic detection techniques adapted to species have to be developed. Moreover it is necessary to keep in mind that some species are easier to detect than others.

5.3.2 Diversity assessment

Diversity assessment of macro-invertebrates are usually used in freshwater to assess and monitor ecosystem health (Oertli et al., 2005; Angélibert et al., 2010). The use of PAM for diversity assessment could be an interesting complement. The link between acoustic communities and macro-invertebrate communities may be relatively direct as demonstrated in the secondary channels of the Rhône (Chapter 3) suggesting that such an approach could be viable.

Diversity assessment with PAM usually evaluate acoustic diversity with acoustic indices. Those methods appeared to need some adjustments to be applied to freshwater ponds due to the low SNR (Chapter 2). To overcome this issue, audio filtering techniques are required. Moreover, a new generation of acoustic diversity indices based on automatic detection of basic elements of the soundscape or sound types seems to be emerging and may be less sensitive to environmental noise (Eldridge et al., 2016).

5.3.3 Hydrological processes

Hydrological processes such as sediment transport and flow turbulence also have specific acoustic signatures. Tonolla et al. (2010) were able to classify riverine habitats characterised by different hydromorphological features according to the amplitude contained in nine frequency bands.

Acoustics appears also as an appropriate method to monitor flow dynamics as demonstrated by field and laboratory experiments (Tonolla et al., 2011, 2009).

Sediment transport measures are usually based on sediment size diversity. This diversity measure is a lagging indicator meaning that it testifies not only of current but also of past sediment transports. Acoustics, on the other hand, could enable to follow instantaneous sediment transport dynamics.

PAM in freshwater habitats could therefore help unravelling spatio-temporal hydrological dynamics.

5.3.4 Biological gas emissions

During this PhD, I found out that the emission of gases during plant respiration or photosynthesis and organic matter decomposition produce whistling and ticking sounds. I have recorded several times in the field the sound of bubbles coming out from the sediments or a plant (Figure 5.4). François Vaillant, an artist and naturalist recorded a video of the phenomenon (https://vimeo.com/125721435).

Unlike in marine environments where O_2 production by the respiration of an algae was monitored with acoustics (Felisberto et al., 2015), this phenomenon has never been reported for freshwater environments in a scientific publication before. Using these sounds could be interesting to monitor those primordial ecosystem processes that are plant respiration and organic mater decomposition. For that, more information on the sound emission mechanisms and conditions are required to better understand this phenomenon and use it further for example to yield information on water chemistry.

5.3.5 Integrating all these sounds: exploiting the components of freshwater soundscapes

The different acoustic elements found in freshwater environments can be informative independently (population, communities, sediment transport and possibly gas emission). Additionally, integrating together all this information would provide an innovative method to assess quickly and non-invasively environmental conditions.

If we look back on the five main key ecological elements described in the *General introduction*, PAM could enable to monitor parts of all five of them (Figure 5.5). First,

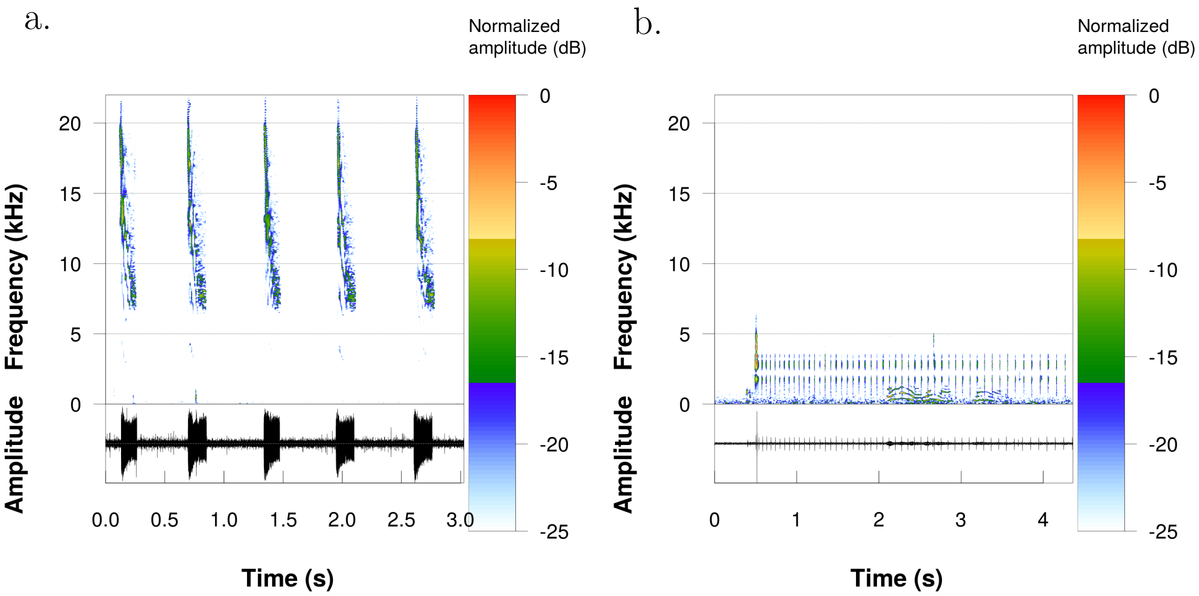

Figure 5.4 – **Spectrogram and oscillograms of plant (a.) and sediment decomposition (b.) sounds** a. Plant respiration sounds recorded on the 6th of August 2015 at 12:30 am in Brégnier-Cordon. b. Sounds of the bubbles emitted by sediment decomposition recorded on the 7th of May 2013 at 3:00 pm in Le Pradet.

biological diversity emitting sounds could be monitored. Flow turbulence is an indicator of hydrological processes which can be monitored by acoustics. Some chemical characteristics of the environment such as O_2 concentration or organic matter content of the sediments may be linked to gas emission sounds. The physical habitat such as the amount of organic debris and sediment transport may also be indicated by gas sounds but also by sediment collision sounds. Finally as demonstrated in the secondary channels of the Rhône (Chapter 3), there is a relationship between connectivity and acoustic communities.

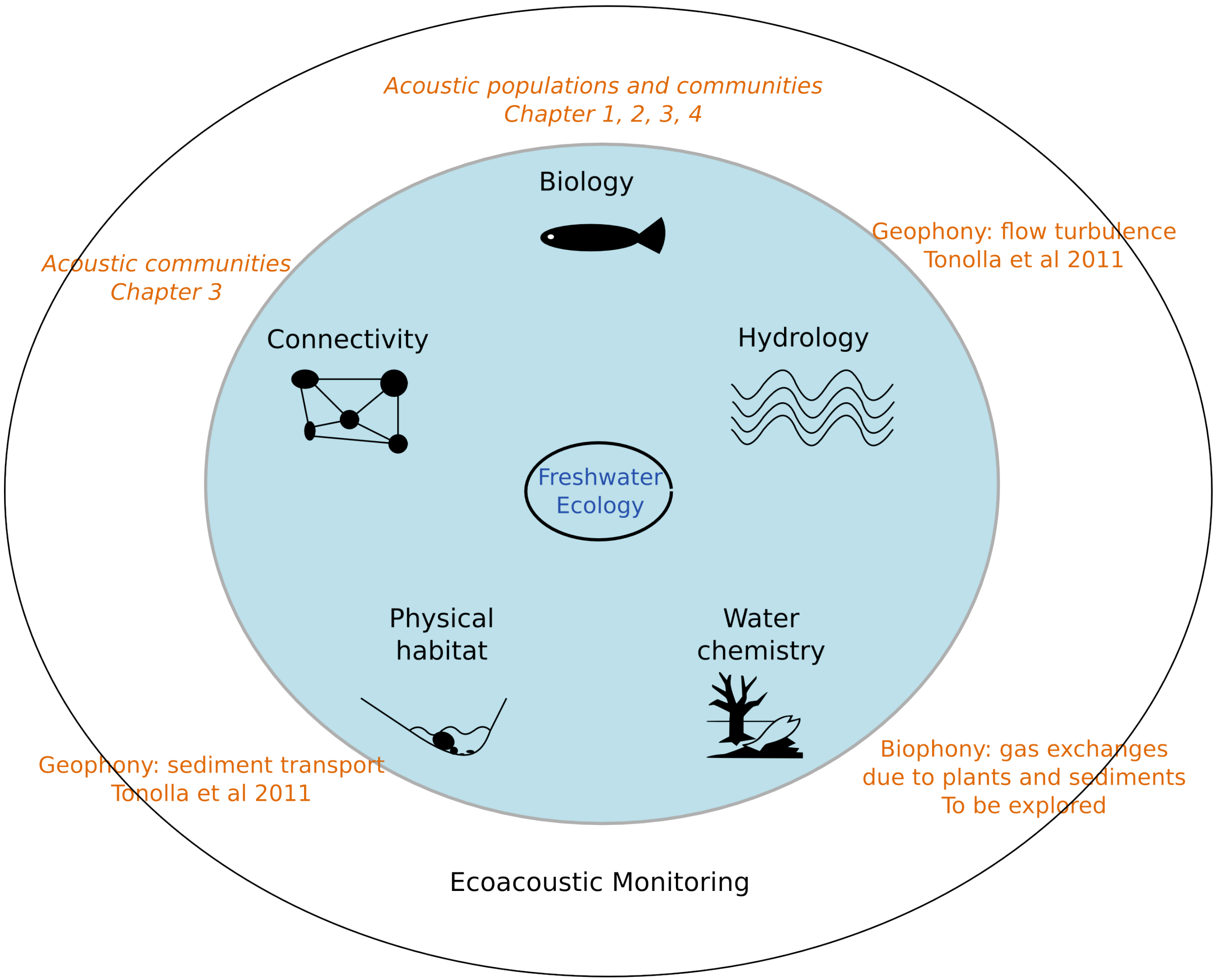

Figure 5.5 – **Summary diagram of ecoacoustic monitoring of ecological processes and factors in freshwater environments**. This diagram shows the five key ecological elements of freshwater environments and ways to monitor them with acoustics.

To integrate all this information, it is necessary to identify and categorise all these acoustic elements and to relate the sounds to animals or processes. There are several main challenges facing freshwater acoustic monitoring which need to be addressed precisely to enable the development of a standardised monitoring method.

5.3.6 Main challenges for ecoacoustic monitoring in freshwater environments

There are five main challenges which need to be addressed to enable efficient use of ecoacoustic monitoring in freshwater environments.

Linking sounds to emitters When a sound is recorded, it is necessary to be able to identify the emitter. In this thesis, I have tried to associate species to sounds by isolating species in aquariums (Chapter 1). This procedure has been moderately successful probably because of the stress induced by laboratory conditions. Working in a larger and naturalised controlled environment such as a small pool with vegetation may yield better results. The sounds of other emitters such as plants and organic matter decomposition also need to be characterised precisely to avoid any confusions. Finally, identifying all the 271 sources in France alone would require years of work, it would therefore be interesting to mutualise the efforts. A freshwater sound library involving a worldwide collaboration of specialists would give momentum to this identification. Such a library would enable a long term conservation of sounds and facilitate information sharing. This mutualisation could be an international project leaded by an international sound library such as the Macaulay library (http://macaulaylibrary.org/) at Cornell University or the sound library of the Muséum National d'Histoire naturelle (https://sonotheque.mnhn.fr/). Indeed, I am currently archiving my recordings at the sonothèque of the muséum.

Sound variability Intra-specific variations in freshwater calls are relatively unknown. It has been shown that some species of Corixidae produce up to five different call types (Aiken, 1982b). Even within one call type, significant variation may be observed, as exemplified by Jansson (1979) on *Arctocorisa carinata* which revealed a geographic variation in their calls. Non-animal sound variability is even more mysterious, plant sounds for example appeared quite variable. This variability has to be estimated and taken into account to enable accurate identification of species and processes.

Temporal variations As demonstrated in this thesis, there are important diurnal variation in species activity. I also found diurnal patterns in gas emission processes which are probably linked to luminosity and temperature. Temporal variations are also observed at the seasonal scale due to annual cycles in species. These temporal variations have to be assessed to know when it is the best time to monitor, according to the study system.

Sound propagation Thanks to the network of hydrophones to record *Micronecta scholtzi* (Chapter 4), we were able to reveal spatial variation in sounds, which could differ significantly even when separated by only 5 meters. Indeed, loud sounds at one hydrophone were sometimes very faint almost undetectable in the next hydrophone, five meters away. Sound propagation in those environments appear relatively complex and understanding spatial interplays would enable to estimate active space which could be useful for PAM for instance.

Links with ecological conditions Deriving links between sounds and ecological condition is one of the main aims of ecoacoustics, however this is not an easy task. Studies along various environmental gradients may increase our understanding. For example studying how chemical conditions influence sound emission by plants could reveal an efficient method to monitor chemical condition or O_2 emission. Additionally, macro-invertebrates have largely been used as water quality indicators. Among the macro-invertebrates sound emitting species are some good indicator species such as Trichoptera and Coleoptera. Detecting the sounds of these species could enable to evaluate the quality of the water. However more knowledge on the link between sound and species is required.

5.4 General conclusions

This PhD thesis is a first exploration of the complexity and dynamism of acoustic populations and communities. The different levels of structure revealed in various freshwater environments suggest an important role of environmental variables in shaping acoustics. However, how the environment applies constraints on acoustic communities and populations are still hypothesised and require formal testing.

We show in this work that there is a significant amount of acoustic diversity in freshwater. This opens several perspectives: i) on an exploratory level, as a large amount of acoustic diversity may still be unraveled , ii) on a fundamental level, as the processes governing assemblage of acoustic population and communities might be understood, and iii) on an applied level since ecoacoustics could enable a better monitoring of these threatened habitats.

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Chapter 6 :

Appendix

Pond in Saron sur Aube (Marne 51), June 2014

6.1 Appendix Chapter 1

6.1.1 Special section proposal accepted in *Freshwater Biology*

Special section: Acoustic methods in freshwater systems: A new frontier in continuous system monitoring

Guest editors : Simon Linke, Camille Desjonqueres, Toby Gifford

Freshwater biodiversity is declining more rapidly than biodiversity in any other realm (Dudgeon et al., 2006). A significant challenge for freshwater conservation is that changes in freshwater ecosystems are not immediately visible. A clear-felled forest is readily apparent, but declines in biodiversity beneath the water surface can go undetected for long periods. This highlights a need for new efficient and instantaneous monitoring tools.

As featured in a recent issue of Science (Servick, 2014), bioacoustics for automated wildlife monitoring has seen a steep increase in interest over the last decade. Since many animals make distinctive sounds, audio field recordings can be used to detect the presence and potentially the density of particular species. Though initial research in wildlife acoustics has focused on birds and mammals, Rountree et al. (2006) predicted ‘with the advent of new acoustic technologies, passive acoustics will become one of the most important and exciting areas of fisheries research in the next decade.’ In fact, sounds produced by many freshwater species have been described in the last three decades, for example fish (Lugli et al., 2003; Colleye et al., 2013; Millot et al., 2011), amphibians (Gerhardt and Huber, 2002), crustaceans (Favaro et al., 2011; Sandeman and Wilkens, 1982) and insects (Aiken, 1985).

Traditional methods for aquatic survey are problematic because: (a) they pose risks to species health and habitat integrity, (b) they can introduce bias due to fright responses in key aquatic species, (c) standard surveying only produces a snapshot of the time and restrained location of surveying – which in many cases does not happen more than once a year and d) monitoring can be very expensive, particularly in areas with remote access. Non-invasive passive acoustic monitoring can address these four problems. A continuous monitoring system with a wide spatial scale – usually impossible for faunal surveys – is extremely desirable since then population trends can be monitored.

Despite the above advantages, bioacoustics for freshwater systems is lagging behind its marine and terrestrial counterparts. While these search terms are far from comprehensive, a Scopus search for ‘bioacoustics and rivers’ yielded a total of 45 hits, while ‘bioacoustics and marine’ and ‘bioacoustics and birds’ yielded 209 and 323 hits respectively. This is partly due to difficulties in matching underwater sounds to the organisms producing them. The classic study by Anderson et al. (2008) for example isolated 62 distinct sounds from the Hudson river. Of these sounds, only four could be identified as known fish sounds, while 21 others were classified as biological, 5 as nonbiological and 32 as unknown.

That said, an ever increasing volume of work is appearing, even in a freshwater setting (Desjonquères et al., 2015; Tonolla et al., 2010; Anderson et al., 2008). Unfortunately, a lot of this work is split between bioacoustics, ecoacoustics, physiology, behavioural science and ecology journals. We see this special section as a 'call to arms' that will bring together interdisciplinary researchers (freshwater conservation and ecology, bioacoustics, signal analysis and physics) showing that acoustic methods to monitor and survey both populations and ecosystems can and should be used in a freshwater setting. For this, knowledge needs to be synthesized – a point to be made in the opening paper by Desjonquères et al. The five remaining papers from France, Ireland and Australia demonstrate the utility of acoustic methods in a variety of settings divided in two approaches:

- a species centered approach at the scale of the population
- a more global approach at the scale of communities or ecosystems. This five papers include acoustic monitoring applications for fish, frogs and invertebrates

We see this special section as having a similar potential to the special issue by Turak and Linke that brought Freshwater Conservation Planning on the map as a scientific discipline and was cited 403 times since 2011.

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6.1.2 Manuscript to be submitted to *Frontiers in Ecology and the Environment*

Real-time Ecosystem Monitoring using Passive Acoustics in Freshwater Environments

Target journal: Frontiers in Ecology and the Environment

Concepts and questions sections: 3000 words & 4-5 tables, figures or text boxes

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Summary

The current biodiversity crisis particularly affecting freshwater environments underlines the urgent need for real-time monitoring devices that could provide updated and reliable ecological information to decision makers. Here, we demonstrate the potential of using new acoustic technologies to monitor freshwater ecosystems. We argue that passive acoustics presents a viable, uninvasive and yet unexplored approach to freshwater ecosystem monitoring, yielding information across three key ecological elements of the freshwater environment, (i) fishes, (ii) invertebrates, and (iii) biophysical habitat.

Recent breakthroughs such as the release of inexpensive low-power computing devices, enable the deployment of automatic recorders to conduct large scale continuous monitoring, hence guaranteeing high spatio-temporal resolution.

We surveyed the substantial but scattered literature on freshwater bio- and ecoacoustics, and justify the need for cross-disciplinary work to mainstream recording and analysis techniques. We conclude that passive acoustics represents a potentially revolutionary development in freshwater ecology, enabling dynamic detection of events to inform conservation practitioners.

In a nutshell

. Freshwater environments represent a global source of biodiversity as well as a primordial reservoir of drinking water. These environments are highly threatened by human activities.

. It is fundamental to monitor efficiently and continuously the health of these environments to inform decision makers and resource managers.

. Acoustic monitoring has recently emerged as a promising and innovative technical tool to monitor whole ecosystems.

. Here, we advocate that acoustic monitoring could constitute a new approach to monitor freshwater environments in a continuous and non-invasive way

Introduction

Freshwater biodiversity is declining more rapidly than biodiversity in any other realm (Dudgeon *et al.* 2006). Catchment protection schemes require to consider both lateral, longitudinal and vertical connectivity implying severe constraints specific to freshwater sampling. Conservation action therefore needs to be carefully planned, while considering spatial and temporal complexity. A second, but potentially unaddressed issue is the lack of immediate visibility of impacts in freshwater ecosystems: declines in biodiversity beneath the water surface can go undetected for long periods. In this paper, we argue that real-time acoustic monitoring has the potential to revolutionise freshwater biodiversity and river health monitoring, by overcoming three key problems with conventional monitoring of aquatic ecosystems (Text Box 1).

Issues with traditional monitoring – the case for acoustic approaches

Traditionally, monitoring in freshwater systems is conducted by collecting samples using techniques such as netting and electrofishing. However, these techniques, as well as other survey techniques for aquatic ecosystems have a number of major drawbacks: they potentially injure the study organism, and they often have low spatial and temporal replication that can bias the outcomes. These sampling methods hence only provide a snapshot at the time of observation rather than a continuous time series that examines the dynamic of the system. The last point especially is a key flaw in traditional techniques – continuous monitoring of aquatic systems is imperative to resolve complex spatio-temporal interplay in riverine processes (Goodman *et al.* 2015).

Panel 1: Problems with Existing Methods in Freshwater Surveys

S Linke and J Bailey during a fish survey in the Yukon river

Problem 1: Risks to ecosystem health and habitat integrity. Classic techniques like netting and electrofishing can cause injury or even death. This is inappropriate for sensitive or threatened species.

Problem 2: Bias. While all sampling methods are biased to varying degrees, a key source of bias is the act of sampling itself. It often causes fright responses, making detection difficult.

Problem 3: **Temporal variation**. Usually single survey events are used to estimate distributions and monitor population. This can only deliver a snapshot of the population at a single sampling time.

Passive acoustic monitoring in aquatic ecosystems, hereafter termed ecoacoustic monitoring (Sueur & Farina, 2015), on the other hand is an uninvasive sampling method that only requires introduction of a hydrophone in the habitat to record the emanating sounds. Spatial and temporal repetition can be easily obtained by placing several hydrophones and setting a proper recording schedule. Finally, detection has no impact on the health of individuals.

Real-time Ecology

The current pace of ecological change is unprecedented and new methods are needed to monitor ecological 'surprises' (Lindenmayer *et al.* 2010). Real-time ecology aims to facilitate understanding of ecosystems undergoing rapid change. In recent years several large projects have championed various forms of real-time ecology. For instance, the North-American NEON (National Ecological Observatory Network) project has set out to design a long-term monitoring project to detect ecological effects of natural and human-induced changes such as climate, land use and invasive species bioacoustics (Keller *et al.* 2008). While not yet analysed, terrestrial sound recorders have been deployed at four NEON sites (Denes *et al.* 2015), realising the potential for continuous monitoring of birds, frogs and insects, and the Remote Environmental Assessment Laboratory (REAL) has implemented several near-real-time monitoring programs (Kasten *et al.* 2012), including terrestrial recording of lake environments. However, real-time continuous monitoring of freshwater environments has not entered any long-term monitoring program.

Although the acoustics component of the NEON program for real-time monitoring is not functional yet, the first scientific studies in real-time ecology are beginning to emerge. Baumgartner *et al.* (2013) for example have achieved real-time detection of several whale species for the purpose of alerting ships to avoid collision and Aide *et al.* (2013) promote real-time acoustic monitoring in order to increase temporal resolution, developing an online platform for automatic processing. Real-time acoustic monitoring in freshwater may benefit numerous applications; for example, monitoring of invasion fronts (see Hu *et al.* 2009 for a terrestrial project monitoring the impact of cane toads), detection of rare species could be linked to other techniques, such as attempts to capture a rare taxon that has been detected in the vicinity; and the capacity for timely alerts to temporally specific events (Colonna *et al.* 2015).

What ecoacoustic monitoring can offer for freshwater ecology

As highlighted in a recent issue of *Science* (Servick 2014) bioacoustics for automated wildlife monitoring has seen an increase in interest over the last decade. Since many animals produce distinctive sounds, audio field recordings can be used to detect the presence of particular species. Though initial research in bio- and ecoacoustics focused on birds and mammals, Rountree *et al.* (2006) predicted "with the advent of new acoustic technologies, passive acoustics will become one of the most important and exciting areas of fisheries research in the next decade."

This paradigm shift has not quite occurred yet; whilst slowly emerging, acoustic monitoring has yet to gain traction in freshwater systems. Although recent and past studies have demonstrated the richness of acoustic communities in freshwater environments (Anderson *et al.* 2008; Desjonquères *et al.* 2015), large knowledge gaps in sound production of freshwater organisms remain, both in ethology and acoustic characterisation.

Acoustic monitoring in aquatic systems is a non-invasive sampling method, thus presenting itself as an alternative to traditional techniques.. Spatial and temporal repetition can be easily obtained by placing several hydrophones and setting a proper recording schedule. Finally, detection has no impact on the health of individuals. Little work has been done on passive acoustic monitoring of freshwater environments, despite its potential to provide significant information on the biology, ecology and population status of aquatic biota through space and time (Koehn and Kennard 2013). This includes information on species' presence or absence, abundance, habitat use (e.g. for feeding or nesting), behaviour (e.g. courtship), and movement biology. We see a role for passive acoustic in all of these, especially processes that require continuous monitoring.

A history of traditional freshwater bioacoustics

While freshwater bioacoustics is commonly seen as an emerging field, it has in fact been an active area of investigation for over 80 years. However, in line with Belovsky *et al.* (2004) who describe ecological research as 'a wave of fashions', freshwater acoustics has seen several waves. For example for fish, serious efforts in cataloguing underwater sounds have been conducted in the late 60s and early 70s when Fish and Mowbray (1970) systematically catalogued fish sounds, both *in situ* as well as recorded in aquaria. At the end of their study the database comprised sounds of 153 marine fish. The world's most comprehensive wildlife sound library is the Macaulay library managed by the Cornell University (http://macaulaylibrary.org). These unique archives store over than 120,000 recordings of birds but only 929 fish recordings.

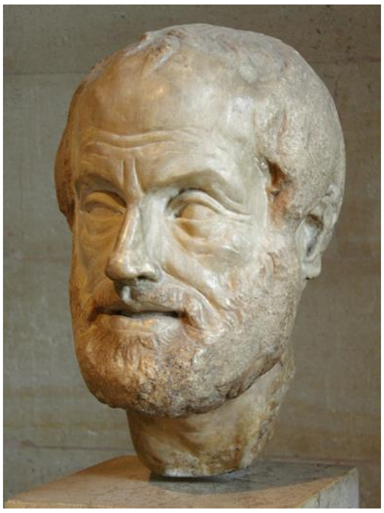
The majority of the 1,800 articles recovered from a Google Scholar search of the key-words "+freshwater+bioacoustics" can be categorised into two groups: (1) mechanisms of biological sound production and behavioural studies, and (2) the effect of anthropogenic noise on aquatic animals. Whilst several articles on aquatic acoustic monitoring do suggest the potential of passive bioacoustics monitoring, these almost exclusively aimed for marine application (primarily marine mammals), and very few had been tested. The application of bioacoustics to freshwater monitoring is therefore virtually non-existent in the literature. However, the following sections will argue a case that aquatic bioacoustics needs to be organised and synthesised so that an ecological application for monitoring purposes becomes possible.

Panel 2: Sonifery in fish as described by Aristotle (Historia Animalium, Book IV, Chapter 4, Translated by D'Arcy Wentworth Thompson)

"No mollusc or crustacean can produce any natural voice or sound. Fishes can produce no voice, for they have no lungs, nor windpipe and pharynx; but they emit certain inarticulate sounds and squeaks, which is what is called their 'voice', as the lyra or gurnard, and the sciaena (for these fishes make a grunting kind of noise) and the caprus or boar-fish in the river Achelous, and the chalcis and the cuckoo-fish; for the chalcis makes a sort piping sound, and the cuckoo-fish makes a sound greatly like the cry of the cuckoo, and is nicknamed from the circumstance.

The apparent voice in all these fishes is a sound caused in some cases by a rubbing motion of their gills, which by the way are prickly, or in other cases by internal parts about their bellies; for they all have air or wind inside them, by rubbing and moving which they produce the sounds.

But in these cases the term 'voice' is inappropriate; the more correct expression would be 'sound'. For the scallop, when it goes along supporting itself on the water, which is technically called 'flying', makes a whizzing `sound; and so does the sea-swallow or flying-fish: for this fish flies in the air, clean out of the water, being furnished with fins broad and long. Just then as in the flight of birds the sound made by their wings is obviously not voice, so is it in the case of all these other creatures. "



Listening for key aquatic groups and processes: Fish, macroinvertebrates and biophysical processes

We will restrict our discussion on ubiquitous sounds by fishes and macro-invertebrates. Some other aquatic groups, such as amphibians, may call underwater but are rarely used as indicators or models in freshwater ecology.

Fishes

Fish – the top of the foodchain in many freshwater ecosystems – are a taxonomic group of economic, ecological and cultural importance. Monitoring fish stocks and communities is thus one of the key tasks in aquatic ecology.. It is surprising that acoustic approaches have not previously used in monitoring, because according to Luczkovich *et al.* (2008) the description of soniferous behaviour in fish assemblages is almost as old as science itself. This is testified by a still quite accurate description by Aristotle of some of the main mechanisms for fish to produce sound (Text Box 2).

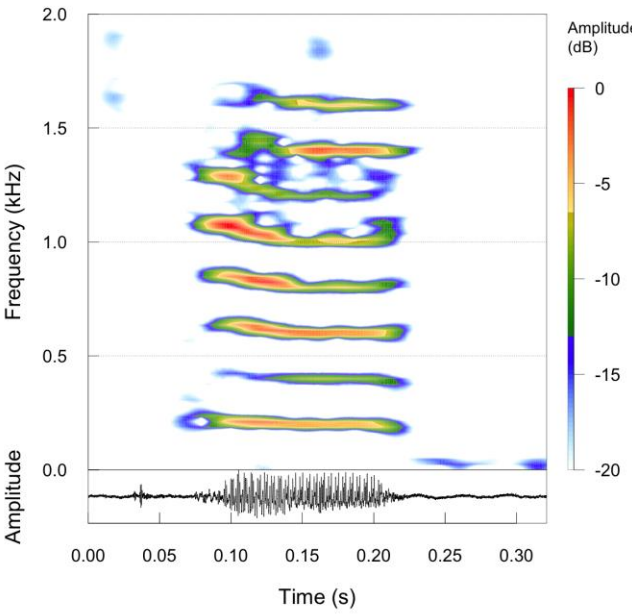
Fine and Parmentier (2015) discuss two main mechanisms of fish sound production. The first class are vibrations of the swimbladder using ‘drumming’ muscles. The second class are stridulatory organs in which the pectoral girdle vibrates or pectoral fins rub against the pectoral girdle as found in the blue catfish, a native species to Eastern Australia (Text Box 3).

The number of 700-800 species of soniferous fish taxa (Rountree *et al.* 2006; Luczkovich *et al.* 2008) is likely to be wildly underestimated (Rountree *et al.* 2006). For instance, in one of the very few comprehensive studies that catalogued and tried to identify all of the sounds in a freshwater survey, Anderson *et al.* (2008) recorded and classified in two sites over 164 recorded hours only four sounds as known fish species, but an additional 21 as biological, as well as 32 as unknown. Unknown/undocumented fish sounds can for example be short clicks, such as the ones characterised by Kottege *et al.* (2015), which will be very hard to include in a monitoring system. This calls for a centralised annotated database of fish sounds to foster monitoring efforts.

Panel 3 : Sound production of the spangled grunter *Leiopotherapon unicolor*

Leiopotherapon unicolor
(picture courtesy of Michael Hammer)

- The spangled grunter (*Leiopotherapon unicolor*) is a soniferous fish in Northern Australia, which emits a distinctive grunt by vibration of its swim bladder, suggesting the possibility of automated passive acoustic population monitoring for this species.
- A typical grunt has been identified as 100-200 ms in duration, containing 3-8 spectral bands with a varying contour.
- There is a clear harmonic structure, with fundamental frequency at 200 Hz, commonly 3-4 strong harmonic overtones, weak second and fifth harmonics, and strong third and fourth harmonics.



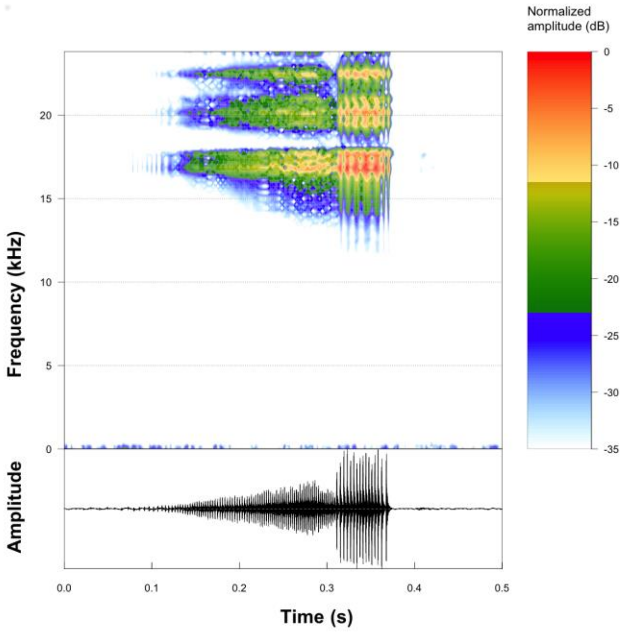
Macroinvertebrates

For a century, aquatic macroinvertebrates (aquatic arthropods, gastropods, molluscs and worms) have been used as indicators in bioassessment and biomonitoring of river health (Norris and Thoms 1999). Identification of insect samples is time consuming and needs considerable expertise by taxonomists. An ecoacoustic approach to river health could use acoustic signals to get an estimate of ecosystem health. While an ecological community approach, that is through the analysis of the sound emanating from a community has rarely been attempted before (Desjonquères et al 2015), numerous studies have described four soniferous aquatic insect orders (Trichoptera, Odonata, Coleoptera and Hemiptera, see Aiken 1985). The main sound production mechanism for aquatic insects is stridulation although there are a few other cases such as air expulsion through the spiracles or flight muscle contraction (Aiken 1985). They produce a wide diversity of sounds with their dominant frequency ranging from 200 Hz to 100 kHz but mainly around 5-6.5 kHz (Aiken 1985, Text Box 4). As Trichoptera, some Coleoptera and Odonata are generally recognised as indicators for healthy ecosystems and Hemiptera on the other hand are often pollution tolerant (Norris and Thoms 1999). There is thus potential to monitor water quality using the species specific sounds of these insect taxa.

Panel 4 : Sound production of *Micronecta griseola*

Mediterranean pond in which the activity of a population of Micronecta scholtzi was monitored acoustically in 2015 with an array of 12 hydrophones

- *Micronecta griseola* is a riverine species of pygmy waterboatman producing distinctive stridulation. Identification based on sound is much easier than that based on morphological characters.
- Micronecta was used as an indicator of water quality in Finnish lakes (Jansson 1987).
- *Micronecta griseola* produces a broadband high pitch sound with the dominant frequency around 17 kHz and a duration of 0.1 second.
- The sound production of this 2 mm species is very conspicuous and detectable at distances of about a few meters.



Biophysical processes

The third component of the aquatic ecosystem is the physical habitat template, of which sound properties have been thoroughly researched in the last decade (Tonolla *et al.* 2011). Physical structure has been quantified by applying emerging remote sensing technologies to measure underwater acoustic patterns in real-time. Unique underwater soundscapes were detected with hydrophones measurements providing a measures of habitat organization. Sounds generated by physical processes reflects important hydraulic (i.e., turbulence levels) and geomorphic (i.e., sediment transport) processes (Tonolla *et al.* 2011).

i) Sediment transport and flow turbulence

Underwater acoustic analysis could be used for rapid spatial surveying of hydraulic conditions including intensity and patterns of sediment transport and hence levels of disturbances in large gravel-bed rivers providing an objective means by which physical habitat can be measured. Underwater sounds produced by flow turbulence and sediment transport are likely to be an important cue to detect suitable habitats for many aquatic organisms (including fish and the adult stage of aquatic insects), as most of them are able to perceive sounds in their environment (Slabbekoorn and Bouton 2008).

Furthermore, habitat classification and characterization using an acoustic approach would be less biased and more cost-effective than traditional observational methods. Acoustic analysis could for example be used to monitor river restoration measures flow characteristics and subsequent sediment transport-deposition processes or to characterize the effect of flow release from hydropower plants (e.g. Lumsdon et al. 2016, in review). Finally, acoustic signals from particle collisions could be used as non-invasive, surrogate means of measuring the intensity of bedload transport and distinguish size fraction transport based on frequency band intensity (Tonolla *et al.* 2011). The ability to assess sediment transport for large gravel-bed rivers remotely and instantaneously, on the floodplain scale, is a missing piece of information important for both basic fluvial geomorphological and ecological understanding as well as for management decisions such as flow release from dams in regulated rivers worldwide.

ii) Gas exchange

Although never reported in scientific publications, plant respiration and organic matter decomposition emit whistling and ticking sounds when the gas bubbles are formed and expulsed. Ecoacoustic monitoring of ecosystem processes has successfully been applied in marine environments to monitor O_2 emissions (Felisberto et al 2015). This phenomenon has been observed in freshwater environments before (supplemental Video S1). Although very promising, these preliminary observations necessitate further research to link water chemistry or gas emissions to distinct sounds.

Vision towards real-time river system ecoacoustic monitoring

In the previous section, we argued that three key elements used in freshwater monitoring can be tracked acoustically. We propose to combine acoustic monitoring of these elements to work towards a system that can observe changes in a river ecosystem in real-time. Until now, continuous monitoring has only been feasible for physical elements of a river, for example through flow gauges, water temperature or water chemistry probes. Spot measurements in field campaigns can monitor all three elements simultaneously (Figure 1). A system that tracks soniferous fishes and macro-invertebrates, hydromorphological changes through time and ecosystem processes could potentially monitor both natural variation, and anthropogenic change. Through continuous recording, it could also factor in diurnal, monthly (lunar), seasonal or annual variation in ecosystem function – variation that is often hard to account for. Such systems have been suggested for terrestrial and marine environments by several authors (Kasten *et al.* 2012; Towsey *et al.* 2012).

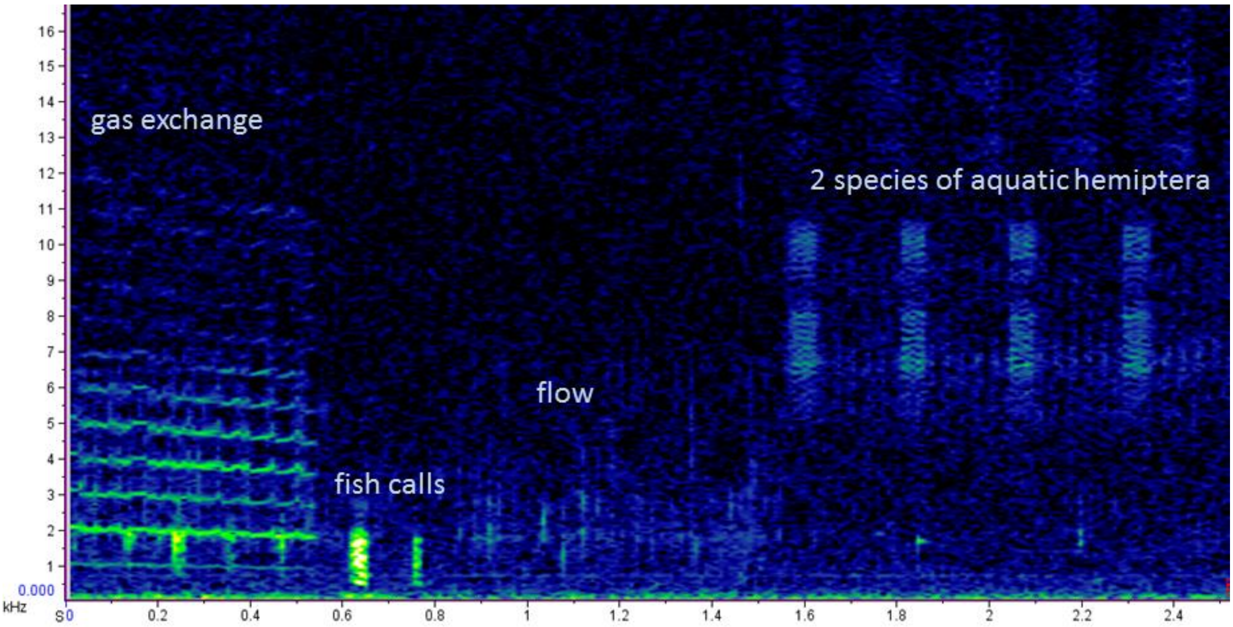


Figure 1 Whole ecosystem monitoring: A spectrogram from the Einasleigh River, North Queensland, Australia featuring sediment gas exchange, fish calls from *Leiopotherapon unicolor*, flow turbulence, and two species of aquatic hemiptera

Developing an operational system for real-time monitoring

In recent years, research on automated detection of various species (Stowell and Plumbley 2014, Ng *et al.* 2011, Fujioka *et al.* 2014) s has been conducted. However only a single study has attempted an automatic detection of a single freshwater species: spotted tilapia, an invasive fish in Australia (Kottege *et al.*, 2015). Data storage capacity has increased exponentially, yet remains a practical issue, particularly for remote portable recording stations (Aide et al 2013). A difficulty until recently with temporally continuous passive acoustic monitoring has been data management, requiring either on-site data storage, or involves severe downsampling of temporal resolution. On-site storage limits the time during which recording can occur without human intervention, is prone to damage from flooding or other unexpected events, and is not real-time; or else requiring real-time transmission from the recording station to the lab, which is prohibitively expensive.

The availability of inexpensive power-efficient microprocessors and widespread high-speed wireless communications networks offers potential solutions to these requirements. By using inexpensive microprocessors, audio processing for real-time detection can be run *in situ*, and high bandwidth audio only stored or transmitted around detection events (Kasten *et al.* 2012). Additionally the lower power consumption of these devices makes long term remote monitoring with solar powered devices feasible.

Future directions towards automatic monitoring systems

Temporal variability in sounds

While temporal variability patterns will emerge once monitoring systems are in place, some background knowledge will be required to separate cyclical or random temporal variation in the activity of soniferous taxa. This is necessary to separate patterns of movement or presence/absence from a site from variations due to calling behaviour schedule. Hardly any literature exists on temporal patterns in the soniferous activity of freshwater organisms. Judging by lessons from the marine field, however, this is an important consideration.

In marine environments, a few studies quantifying temporal variations have emerged in the last ten years. Similar patterns were described two distinct diurnal soundscapes on the coast of South-Africa including 37 unique sound patterns (Ruppé *et al.*, 2015). Sonic similarity varied between day and night patterns – during the day similar sounds occurred at the same time whereas nocturnal sounds were spectrally separated.

More complex patterns can be found as reported in a single species such as the Atlantic Cod (Rowe and Hutchings 2006). In this case, not only a diel variation, as well as seasonal variation, but also an interaction between diel variation and location were identified. All of these patterns will pose obstacles for automatic monitoring and demonstrate that an understanding of the underlying ecological mechanisms will be mandatory to operationalise a monitoring scheme.

Deriving links between sounds and system health

A recent trend in acoustics is the development of ‘holistic’ acoustic approaches as promoted by ecoacoustics (Sueur & Farina, 2015), in which environmental acoustic recordings are analysed as a whole, rather than trying to isolate component biotic sounds as has been historically typical in bioacoustics. Various acoustic indices have been related to other environmental indicators; particularly various measures of acoustic diversity, richness or complexity as proxies for biodiversity (Sueur *et al.* 2014; Parks *et al.* 2014; Towsey *et al.* 2014) as well as habitat heterogeneity (Tonolla *et al.* 2011).

The relationship between acoustic richness and ecosystem health is neither straightforward nor universal. Whilst in some circumstances richer sounds may indicate greater biodiversity and healthier habitat, in other circumstances the opposite may be true. For example soniferous invertebrate abundance can be an indicator of both good and bad ecosystem health, as described above. Furthermore substantial temporal variation and spatial microvariation in underwater acoustics has been shown (Desjonquères *et al.*, 2015), arguing against any simple relationship between acoustic richness and ecosystem health. While a recent reef study, found a correlation between fish richness and an index of acoustic complexity (Pieretti and Farina 2013), Staaterman *et al.* (2013) described that variation in acoustic complexity could mainly be attributed to the presence or absence of snapping shrimp – a ubiquitous but diurnal animal. This highlights that more research is in this field is needed before appropriate metrics and robust recording protocols can be established.

Conclusion

The time is ripe for real-time whole ecosystem monitoring. Ecoacoustic monitoring offers a feasible and cost-effective method, for freshwater applications. There appears to be plenty of information about the ecosystem obtainable through acoustic measurements – from all trophic levels of biota as well as the physical environment. A vast array of knowledge about acoustics in freshwater systems exists, however this knowledge is fragmented, across pockets of ecology, acoustics, biology and conservation planning. While underwater sounds are present in repositories like the Cornell sound archive, the majority of the records are decades old. For example a search for *Actinopterygii* (Ray-finned fishes) returned 961 records (24 Nov 2015), of which only eight records were recorded in the 21st century. This is in contrast to the published literature – clearly active bioacoustics research is being conducted across many spatial domains and with a multitude of purposes. For passive acoustics to play a major role in conservation science, this knowledge needs to be assembled in one repository, where catalogued sounds are linked to proper metadata (see Roch *et al.* 2013 for a proposed metadata structure). Bird-DB is an example in terrestrial bioacoustics. Such a repository can then be linked to environmental and other biodiversity data from GBIF (Global Biodiversity Information Facility, Flemons *et al.* 2007) or similar repositories to enable proper synthesis and analysis. This could potentially speed up identification of unknown sounds in aquatic systems greatly, thus facilitating a pathway to an ecoacoustic monitoring approach.

We see this paper as a call to action for freshwater ecoacousticians, as well as the wider freshwater research community to share resources and contribute to a shared knowledge base. This knowledge base could transform freshwater ecoacoustics into an operational discipline for whole-ecosystem monitoring beyond single species studies within the next 10 to 15 years.

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6.2 Appendix Chapter 2

Table 6.1 – **Main characteristics of the three ponds sampled.**

	Pond 1	**Pond 2**	**Pond 3**
Geographical position	"48°34.523'N, 1°53.341'E"	48°40.560'N, 1°55.865'E	48°40.772'N, 1°55.840'E
Type of habitat	closed	open	semi-closed
Altitude (m)	168	181	177
Depth at 1m from the shore (m)	0.35	0.32	0.38
Perimeter (m)	174	85	170
Nature of the sediment	Silt and clay	silt	silt
Hydrophytes (submerged)*	0	0	0.5
Helophytes*	0	0.5	0
pH	6.68	7.62	6.75
$[PO4]$ (mg/L)	0.25	0	0.20
$[NO2]$ (mg/L)	0.007	0	0

* Frequency of hygrophytes and helophytes on 10 points equally distributed along the perimeter of the pond (Le Viol et al., 2009).

6.3 Appendix Chapter 3

Table 6.2 – **Acoustic characteristics of the 128 recorded sound types: Category, number of sounds on which the measures are based, dominant frequency and duration given as mean ± s.d.** See Table 3.1 for category description.

Sound type	**Category**	**Number of measured recordings**	**Dominant frequency (± kHz)**	**Duration (± s)**
6	1. Pure tone	1	3.871 ± 0	0.021 ± 0
15	1. Pure tone	6	7.21 ± 2.302	2.852 ± 3.117
39	1. Pure tone	6	2.566 ± 0.282	0.4 ± 0
51	1. Pure tone	6	0.502 ± 0.025	0.35 ± 0.141
65	1. Pure tone	3	15.469 ± 0.211	0.259 ± 0.036
67	1. Pure tone	1	1.411 ± 0	0.955 ± 0
83	1. Pure tone	3	5.4 ± 0.004	0.509 ± 0.16
95	1. Pure tone	6	9.3 ± 0.871	0.854 ± 0.608
101	1. Pure tone	6	0.973 ± 0.059	0.135 ± 0.068
104	1. Pure tone	6	8.934 ± 0.657	0.144 ± 0.025
121	1. Pure tone	4	4.91 ± 0.223	0.336 ± 0.349
126	1. Pure tone	6	4.563 ± 0.547	0.053 ± 0.007
4	2. Noisy sound	5	12.068 ± 6.688	0.015 ± 0.001
12	2. Noisy sound	6	3.19 ± 0.114	1.147 ± 0.368
13	2. Noisy sound	1	2.408 ± 0	0.085 ± 0
21	2. Noisy sound	6	0.365 ± 0.29	0.274 ± 0.1
23	2. Noisy sound	6	1.263 ± 0.463	0.288 ± 0.184

Sound type	**Category**	**Number of measured recordings**	**Dominant frequency (± kHz)**	**Duration (± s)**
26	2. Noisy sound	3	2.027 ± 0.626	0.018 ± 0.003
42	2. Noisy sound	6	1.952 ± 0.212	0.302 ± 0.082
43	2. Noisy sound	6	1.376 ± 1.279	0.228 ± 0.066
45	2. Noisy sound	6	0.128 ± 0.049	10 ± 0
46	2. Noisy sound	6	16.724 ± 1.24	0.1 ± 0.048
52	2. Noisy sound	2	10.057 ± 0.153	0.052 ± 0.02
77	2. Noisy sound	6	3.652 ± 0.594	0.048 ± 0.034
89	2. Noisy sound	6	1.373 ± 0.096	0.8 ± 0.433
90	2. Noisy sound	6	6.207 ± 3.121	0.252 ± 0.093
91	2. Noisy sound	1	0.946 ± 0	0.213 ± 0
93	2. Noisy sound	6	2.037 ± 0.741	0.101 ± 0.059
99	2. Noisy sound	3	4.293 ± 0.077	0.126 ± 0.033
103	2. Noisy sound	6	2.316 ± 0.478	0.136 ± 0.036
105	2. Noisy sound	6	7.111 ± 1.231	0.316 ± 0.094
106	2. Noisy sound	6	1.151 ± 0.753	0.125 ± 0.03
107	2. Noisy sound	2	4.021 ± 0.905	0.554 ± 0.223
109	2. Noisy sound	6	2.487 ± 0.715	0.198 ± 0.072
120	2. Noisy sound	1	12.972 ± 0	0.134 ± 0
124	2. Noisy sound	1	7.32 ± 0	0.149 ± 0
7	3. Simple pulse	6	3.687 ± 2.211	0.016 ± 0.001
11	3. Simple pulse	6	7.397 ± 3.395	0.018 ± 0.002
18	3. Simple pulse	1	1.468 ± 0	0.39 ± 0
25	3. Simple pulse	6	12.639 ± 3.659	0.014 ± 0.001
44	3. Simple pulse	6	4.071 ± 0.492	0.018 ± 0.002
64	3. Simple pulse	6	1.429 ± 1.191	0.016 ± 0.001
75	3. Simple pulse	6	4.95 ± 2.881	0.018 ± 0.004
1	4. Composed pulse	6	3.484 ± 0.325	0.116 ± 0.007
10	4. Composed pulse	1	5.734 ± 0	0.481 ± 0
14	4. Composed pulse	4	8.212 ± 2.85	1.184 ± 0.672
16	4. Composed pulse	6	8.049 ± 1.516	3.051 ± 3.671
17	4. Composed pulse	6	13.387 ± 2.082	0.75 ± 0.514
27	4. Composed pulse	6	9.484 ± 0.766	1.428 ± 0.828
29	4. Composed pulse	6	4.51 ± 1.76	0.367 ± 0.067
31	4. Composed pulse	6	3.413 ± 0.085	0.156 ± 0.024
36	4. Composed pulse	1	6.245 ± 0	0.91 ± 0
37	4. Composed pulse	6	9.771 ± 2.162	0.051 ± 0.019
38	4. Composed pulse	6	11.566 ± 0.63	0.196 ± 0.021
49	4. Composed pulse	6	8.796 ± 4.544	0.323 ± 0.092
53	4. Composed pulse	6	8.989 ± 3.344	0.296 ± 0.078
56	4. Composed pulse	4	15.596 ± 0.08	1.434 ± 0.443
58	4. Composed pulse	6	10.539 ± 3.72	0.286 ± 0.179
60	4. Composed pulse	6	5.181 ± 2.606	1.172 ± 1.071
61	4. Composed pulse	6	10.117 ± 0.216	0.102 ± 0.078
66	4. Composed pulse	6	4.008 ± 1.176	0.383 ± 0.036

Sound type	**Category**	**Number of measured recordings**	**Dominant frequency (± kHz)**	**Duration (± s)**
68	4. Composed pulse	6	10.68 ± 2.027	0.203 ± 0.051
69	4. Composed pulse	6	3.007 ± 0.594	0.36 ± 0.065
70	4. Composed pulse	5	3.678 ± 0.507	0.025 ± 0.001
71	4. Composed pulse	6	8.457 ± 5.079	0.178 ± 0.067
72	4. Composed pulse	6	10.187 ± 0.156	0.124 ± 0.075
73	4. Composed pulse	6	3.453 ± 3.008	0.606 ± 0.426
76	4. Composed pulse	4	3.192 ± 0.761	0.132 ± 0.015
78	4. Composed pulse	6	2.051 ± 0.646	10 ± 0
81	4. Composed pulse	1	2.683 ± 0	2.044 ± 0
82	4. Composed pulse	6	3.728 ± 0.394	0.354 ± 0.291
85	4. Composed pulse	6	1.663 ± 0.112	0.172 ± 0.176
86	4. Composed pulse	1	12.651 ± 0	2.431 ± 0
94	4. Composed pulse	6	7.446 ± 1.791	0.174 ± 0.084
96	4. Composed pulse	1	2.249 ± 0	0.332 ± 0
97	4. Composed pulse	1	1.043 ± 0	0.112 ± 0
98	4. Composed pulse	6	5.186 ± 1.834	0.094 ± 0.048
100	4. Composed pulse	6	3.967 ± 0.253	0.215 ± 0.023
102	4. Composed pulse	2	6.237 ± 2.791	0.597 ± 0.52
108	4. Composed pulse	6	1.633 ± 0.003	0.108 ± 0.07
110	4. Composed pulse	4	4.064 ± 0.134	0.064 ± 0.019
111	4. Composed pulse	6	10.702 ± 1.462	0.244 ± 0.139
113	4. Composed pulse	2	3.492 ± 0.086	4.172 ± 0.769
116	4. Composed pulse	3	3.199 ± 0.042	0.288 ± 0.021
117	4. Composed pulse	1	1.922 ± 0	0.486 ± 0
119	4. Composed pulse	6	6.431 ± 0.302	0.256 ± 0.064
125	4. Composed pulse	1	5.608 ± 0	2.275 ± 0
127	4. Composed pulse	1	3.728 ± 0	0.476 ± 0
9	5. Harmonic sound	6	3.428 ± 0.234	0.275 ± 0.248
20	5. Harmonic sound	1	13.682 ± 0	0.052 ± 0
24	5. Harmonic sound	6	13.232 ± 0.199	0.109 ± 0.033
28	5. Harmonic sound	4	9.056 ± 0.02	0.612 ± 0.002
30	5. Harmonic sound	3	9.15 ± 0.108	0.043 ± 0.007
32	5. Harmonic sound	2	15.178 ± 0.185	0.169 ± 0.05
33	5. Harmonic sound	6	9.44 ± 2.456	0.117 ± 0.03
34	5. Harmonic sound	6	9.404 ± 1.035	0.928 ± 0.554
35	5. Harmonic sound	6	6.562 ± 1.571	0.062 ± 0.019
40	5. Harmonic sound	6	2.36 ± 0.13	0.115 ± 0.05
47	5. Harmonic sound	6	2.613 ± 0.109	0.107 ± 0.023
50	5. Harmonic sound	6	6.09 ± 2.334	0.805 ± 0.34
55	5. Harmonic sound	6	8.37 ± 0.444	0.11 ± 0.068
84	5. Harmonic sound	6	7.406 ± 2.582	1.506 ± 0.334
88	5. Harmonic sound	6	8.77 ± 1.345	0.03 ± 0.012
114	5. Harmonic sound	1	3.276 ± 0	3.738 ± 0
115	5. Harmonic sound	6	0.564 ± 0.002	0.451 ± 0.12

Sound type	**Category**	**Number of measured recordings**	**Dominant frequency (± kHz)**	**Duration (± s)**
122	5. Harmonic sound	2	15.431 ± 3.111	0.251 ± 0.089
2	6. Irregular sound	6	0.162 ± 0.149	0.046 ± 0.033
3	6. Irregular sound	6	0.686 ± 0.428	0.023 ± 0.005
5	6. Irregular sound	6	1.339 ± 0.8	0.018 ± 0.001
8	6. Irregular sound	6	0.232 ± 0.126	0.1 ± 0.028
41	6. Irregular sound	6	3.206 ± 1.451	2.361 ± 0.972
54	6. Irregular sound	6	3.219 ± 2.987	0.022 ± 0.006
57	6. Irregular sound	6	0.2 ± 0	10 ± 0
62	6. Irregular sound	6	0.654 ± 0.44	10 ± 0
63	6. Irregular sound	6	7.652 ± 4.687	0.037 ± 0.03
74	6. Irregular sound	6	0.497 ± 0.159	10 ± 0
79	6. Irregular sound	5	0.2 ± 0.037	4.025 ± 3.235
92	6. Irregular sound	6	5.666 ± 4.934	0.832 ± 0.042
19	7. Composed sound	6	2.081 ± 1.007	2.545 ± 1.42
22	7. Composed sound	6	3.731 ± 2.142	0.162 ± 0.116
48	7. Composed sound	4	11.158 ± 0.125	6.896 ± 4.602
59	7. Composed sound	6	2.096 ± 0.635	0.609 ± 0.225
80	7. Composed sound	6	9.933 ± 1.784	3.231 ± 1.225
87	7. Composed sound	6	6.449 ± 0.117	1.394 ± 0.135
112	7. Composed sound	6	5.083 ± 0.03	5.276 ± 0.363
118	7. Composed sound	6	5.644 ± 0.144	4.204 ± 2.22
123	7. Composed sound	1	13.323 ± 0	0.212 ± 0
128	7. Composed sound	1	2.405 ± 0	10 ± 0

Table 6.3 – **Environmental variables in the six sites monitored**: geographic location, temperature, water level variation and lateral connectivity index.

Site	**Geographical position (N/E)**	**Mean temperature (°C)**	**Mean absolute daily temperature deviation (°C)**	**sd of the water level (mm)**	**Connectivity**
BEAR	45°46’34.118” / 5°46’43.324”	15.87	0.26	9.14	0.20
GRAN	45°37’45.647” / 5°38’41.029”	15.34	0.23	4.33	0.77
MOIR	45°47’27.342” / 5°46’57.074”	12.48	0.02	16.06	0.50
MORT	45°40’1.94” / 5°35’28.952”	19.71	0.70	13.14	0.15
ROSS	45°38’13.867” / 5°36’57.227”	17.90	0.5	16.93	0.32
VILO	45°38’1.858” / 5°37’10.301”	18.60	0.64	42.62	0.043

Table 6.4 – **Random intercept values for the sites in the models 1 and 3**. The models were Generalized Linear Mixed Models (GLMM) designed to analyse the relationship between the acoustic composition of the sites and the environmental variables.

Site	**Random intercept model 1**	**Random intercept model 3**
BEAR	0.77	0.31
GRAN	0.14	0.005
MOIR	-0.45	-0.07
MORT	-0.16	-0.03
ROSS	0.01	-0.01
VILO	-0.31	-0.25

Ecologie et diversité acoustique des milieux aquatiques : exploration en milieux tempérés

Résumé : Une grande diversité d'animaux produit des sons pour communiquer, s'orienter, ou lors de la réalisation d'actes comportementaux comme la prise de nourriture. Ces sons ne se répartissent pas aléatoirement dans l'espace et le temps suggérant l'existence de règles d'assemblage sonore qui structurent les populations et communautés acoustiques. Les environnements d'eau douce, et en particulier les mares, sont considérés comme les réservoirs d'une importante diversité biologique, et donc potentiellement abritant un nombre significatif d'espèces produisant des sons. Cependant la diversité acoustique de ces milieux naturels n'a jamais été explorée.

L'objectif principal de cette thèse est d'explorer pour la première fois la diversité acoustique présente dans les milieux d'eau douce en climat tempéré en étudiant les structures des populations et communautés acoustiques et en explorant les processus pouvant déterminer ces structures.

Une revue bibliographique sur la production sonore par les organismes d'eau douce ainsi que des enregistrements d'espèces cibles effectués en laboratoire révèlent qu'une diversité acoustique particulière existe dans les environnements d'eau douce en milieux tempérés. Pour comprendre comment cette diversité est structurée, les communautés acoustiques de trois mares situées dans des environnements différents ont été enregistrées et suivies au cours du temps. Cette étude révèle que les trois mares sont caractérisées par des communautés acoustiques riches et distinctes ayant des dynamiques spatio-temporelles spécifiques. Les facteurs potentiels structurant les communautés acoustiques d'eau douce ont été recherchés en testant si la composition de communautés acoustiques dans six bras morts de la plaine d'inondation du Rhône était liée à des variables environnementales. Nos résultats montrent que les communautés acoustiques des bras morts sont significativement liés à une variable environnementale: le degré de connectivité entre les bras morts et le lit principal de la rivière. Ce résultat suggère un rôle clé de cette variable dans les règles d'assemblage des communautés. Enfin, pour comprendre les processus possibles liant la production de sons et l'environnement naturel, une population de l'insecte aquatique *Micronecta scholtzi* a été suivie par des enregistrements acoustiques dans une mare méditerranéenne. Le niveau d'activité acoustique de *M. scholtzi* a été estimé de façon continue à l'aide d'un réseau de 12 capteurs sonores synchronisés. L'activité acoustique était caractérisée par un rythme circadien, dont les propriétés étaient perturbées par la diffusion expérimentale d'un bruit d'origine anthropique. Cette expérience révèle que les effets de la pollution sonore peuvent être observés à l'échelle d'une population d'insectes aquatiques.

Ce travail montre ainsi l'existence d'une diversité acoustique dans les milieux d'eau douce et identifie des relations entre production acoustique et facteurs environnementaux. Ce travail ouvre également des perspectives intéressantes d'utilisation de l'acoustique pour aborder des problématiques d'écologie fondamentale et appliquée en milieu d'eau douce.

Mots clés : milieux aquatiques d'eau douce, bioacoustique, suivi de biodiversité, sons subaquatiques, communauté acoustique.

Acoustic diversity and ecology of freshwater environments: exploration in temperate environments

Abstract: An important diversity of animal species produces sounds during communication, orientation, movement, or prey-predator acts. These sounds are not distributed randomly in space and time and are therefore thought to follow assembly rules forming either acoustic populations or acoustic communities. Freshwater environments, and ponds in particular, are considered as primary resources for biological diversity and as such host a potentially significant number of soniferous species. However the acoustic diversity of these natural environments remains totally unexplored.

The main aim of this PhD was to explore for the first time the acoustic diversity found in temperate freshwater by studying the patterns and structural processes of a selection of acoustic populations and communities recored in several types of freshwater environments.

A review of the literature on sound production by freshwater organisms along with laboratory recordings of target species revealed that a valuable acoustic diversity can be found in temperate freshwater environments. To understand how the acoustic diversity is structured, the acoustic communities of three temperate ponds were acoustically monitored. This study revealed that the three ponds were characterised by rich and distinct acoustic communities with specific spatio-temporal dynamics. To further understand the potential factors structuring freshwater acoustic communities, environmental variables were assessed along with the composition of acoustic communities found in six secondary channels of the Rhône riverine floodplain. Two environmental variables were investigated: the water temperature and the level of lateral connectivity of the secondary channels to the main river. Acoustic communities in the Rhône riverine floodplain were clearly structured by lateral connectivity suggesting a role of this key variable as an assembly rule. Finally to understand the possible processes linking animal acoustics and the natural environment, a population of aquatic insect, *Micronecta scholtzi*, was acoustically monitored in a Mediterranean pond. The level of *M. scholtzi* acoustic activity was assessed continuously using a network of twelve synchronised acoustic sensors. The acoustic activity of *M. scholtzi* showed a regular daily pattern that was modified in amplitude and phase by the playback of an anthropogenic noise. This experiment revealed that the effects of noise pollution may emerge at an aquatic insect population level.

This PhD unraveled the existence of a significant amount of unexplored acoustic diversity in freshwater environments and identified links between acoustics and the environment. This research opens interesting perspectives in the use of acoustic to tackle fundamental and applied ecological questions in freshwater environments.

Keywords: freshwater environments, bioacoustics, biodiversity monitoring, underwater sounds, acoustic community.

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