Chapter 5

Bioacoustics approaches in biodiversity inventories

by

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Abstract

Acoustic emissions of animals serve communicative purposes and most often contain species-specific and individual information exploitable to listeners, rendering bioacoustics predestined for biodiversity monitoring in visually inaccessible habitats. The physics of sound define the corner stones of this communicative framework, which is employed by animal groups from insects to mammals, of which examples of vocalisations are presented. Recording bioacoustic signals allows reproducible identification and documentation of species' occurrences, but it requires technical prerequisites and behavioural precautions that are summarized. The storing, visualizing and analysing of sound recordings is illustrated and major software tools are shortly outlined. Finally, different approaches to bioacoustic monitoring are described, tips for setting up an acoustic inventory are compiled and a key for procedural advancement and a checklist to successful recording are given. Extensive literature and reference to a collection of web resources (http://www.bioacoustics.myspecies.info) complete the text.

Key words: acoustic, communication, vocalisation, sound, echolocation, biodiversity monitoring, wildlife recording

1. Introduction

Classification of animals observed or collected for biological inventories predominantly relies on visual attributes. However, many animals generate acoustic signals for communication and orientation, which are predestined for eavesdropping on their presence and behaviour. Acoustic signals can be received over varying distances, allowing for unobtrusive detection and observation of their producers. Acoustic observations are well established for *e.g.* birds, insects, anurans, bats or whales. Depending on type of signals and taxonomic group, species identification, abundance estimation or behavioural assessment is possible. But physical properties of sound require certain precautions during recording, analysis as well as interpretation. We outline these prerequisites, describe types of bioacoustical signals for major taxonomic groups, and present a short review on state-of-the-art equipment and methods for bioacoustic recording and analyses. We sum up with a step-by-step key on how to proceed in bioacoustic inventories and research.

2. Physics of sound

Sound consists of oscillating pressure waves travelling at temperature-dependent speed through media like air (343 m/s at 20°C), water (1484 m/s at 20°C) or the ground (~5000 m/s depending on porosity). The number of cycles per second indicates sound frequency and is measured in Hertz (Hz). The frequency range of human hearing ranges approximately from 20 Hz to 20 kHz, and is anthropocentrically considered as 'audible sound'. But hearing ranges of most animals extend below or above this human hearing range. Signals below are termed infrasound and are not recordable with standard equipment. Infrasound waves travel long distances and are well documented for seismic or weather events, but they are also generated and perceived by elephants or whales for long distance communication. Signals above human hearing range are termed ultrasound and used mainly for echolocation by bats and dolphins.

Sound energy is usually not measured as peak pressure but as the square Root of the Mean of the Squared pressure (RMS), because this quantifies the energy over all waveforms in a signal. It is most sensible to indicate this RMS pressure not as N/m^2 but rather on a logarithmic scale, which better corresponds to increments of perceived sensation. Sound pressure is therefore indicated as the ratio of pressure P to a reference pressure P_0 on a logarithmic scale. The commonly used reference pressure P_0 is $2x10^{-5}$ N/m^2 RMS or 20 µpascals RMS. This corresponds to a sound intensity of 10^{-12} $Watt/m^2$ and is roughly equal to the lowest pressure humans can detect at 1000 Hz. The log of the ratio, termed Bel, is divided by 10 and expressed in decibel (dB), to achieve sensible numbers. Because intensity varies as the square of the pressure, levels referring to the above reference are expressed as 20 times the $\log_{10}$ of the ratio of P/P_0 and expressed as dB, thus sound pressure level (dB) = $20 \log_{10} (P/P_0)$. The logarithmic scale facilitates calculations within the wide range of intensities in sensory physiology – while a 3 dB difference is just perceptible, it takes about 10 times the intensity to sound twice as loud. Sound intensity decreases with the square of the distance due to spherical spreading loss. Thus, doubling of the distance leads to an intensity level drop to a quarter, or a change of -6 dB. Equally, sound pressure level drops by 6 dB when doubling the distance (Sengpiel, 2010). As dB measures in water refer to a reference pressure of 1µpascal, all measures in water are 26 dB higher than in air for an identical sound pressure.

Furthermore, sound attenuation additionally increases progressively with increasing frequency due to atmospheric absorption (Lawrence & Simmons 1982), basically limiting *e.g.* ultrasound echolocation of bats to short ranges (~5 to 50 m depending on signal characteristics). Ultrasound becomes more directional with increasing frequency, which can additionally influence perceived signal characteristics. Sounds carry through dense vegetation, over considerable distances, and in darkness, rendering acoustics a non-invasive and economic way to study *e.g.* marine mammals, hidden forest inhabitants or nocturnal animals.

Recording of sounds requires a microphone (or a hydrophone), transducing mechanical energy from sound pressure into electrical voltage. Different frequency ranges and media require appropriate microphones, particularly for ultrasound and underwater sound recording (see Technologies section).

3. Sound producing animals

Animals produce sounds for territorial defence, for group interactions, mate attraction and for orientation. Most vocalisations exhibit highly distinctive features, to be used in taxonomy and systematics, and thus biodiversity research. Several new species have been discovered by their distinct signals, *e.g.* secretive and nocturnal species or morphologically similar (cryptic) sibling species. Bioacoustic monitoring is widely applied for well-known taxonomic groups like birds and mammals, but its application is now extended into lesser-known, species-rich groups such as insects. In the following, major taxonomic groups hitherto studied by bioacousticians are briefly characterised:

3.1. Insects

Most research concentrated on the Cicadidae and Orthoptera (*e.g.* Diwakar *et al.,* 2007; Riede, 1997; Sueur, 2006), a fraction of insects that produce loud audible songs (Fig. 1 A-C). Many more insect groups produce ultrasounds or weak vibrational signals not perceptible to man. Using appropriate microphones and amplifiers, acoustic inventorying and monitoring could easily be extended to other target groups, communicating by vibration (*e.g.* treehoppers: Hemiptera: Membracidae; Cocroft & McNett, 2006) or underwater stridulation, as documented for water bugs (Jansson, 1973). Sounds of insects are species-specific and stereotyped, but recognition of species-specific features requires visualisation. The temporal structure of their songs varies with temperature, further aggravating the recognition of insect species in the field.

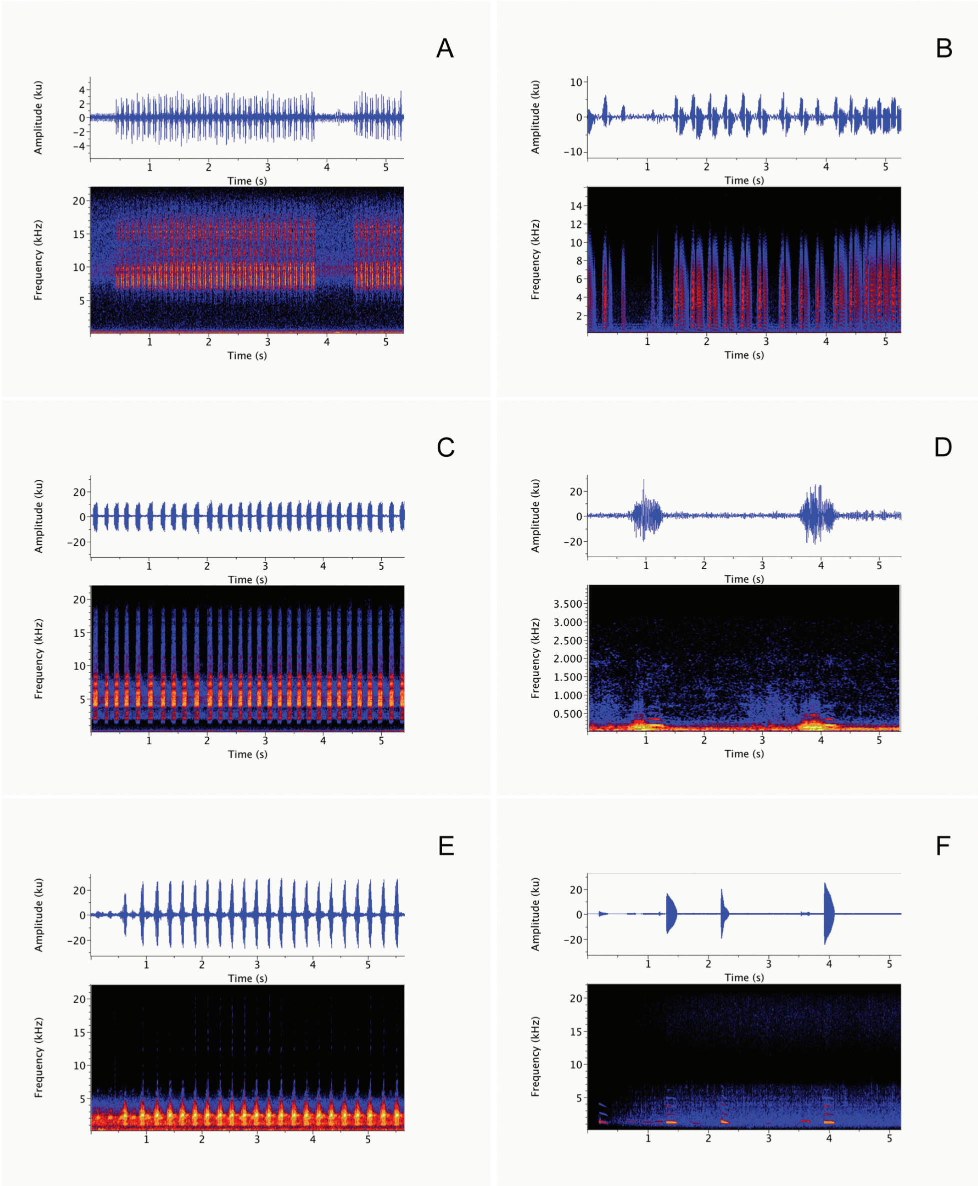


Fig. 1. Amplitude and spectrographic displays of acoustic signals of insects, fish, and anurans. A. Great Green Bush Cricket (*Tettigonia viridissima*); B. Scarabaeid Beetle (*Copris incertus*); C. Mediterranean Cicada (*Cicada orni*); D. Italian Freshwater Goby (*Padogobius martensi*); E. European Tree Frog (*Hyla arborea*); F. Common Midwife Toad (*Alytes obstetricans*). Note the different frequency scales and dB ranges not comparable between subplots! Spectrograms were generated from recordings of the authors except A taken from data recorded at 18°C, available from http://www.biologie.uni-ulm.de/cgi-bin/soundobj.pl?id=32797&lang=e&sid=T (Digital Orthoptera Specimen Access DORSA archives – http://www.dorsa.de).

Acoustic signals used in mate finding have the potential for speciation effects, and enable bioacousticians to find new species. Particularly in insects, striking differences in song structure of morphologically similar species helped taxonomists to diagnose and describe ‘cryptic species’, many of which cannot be differentiated without a sound recording. Walker (1964) reviewed studies on songs and taxonomy of North American Orthoptera. He found that most morphologically defined species consisted of complexes of cryptic species. He estimated that one-fourth of the gryllid and tettigoniid species of the eastern USA had never been recognized or had been wrongly synonymized (Walker, 1964: 346). In Europe, acoustic analyses led to the discovery of new and important information about the biogeography of *Cicadetta* species (Sueur & Puissant, 2007).

3.2. Fish

Sound production in fish is poorly studied, although common: more than 50 Teleost families include sound producing species (Fig. 1D). Fish produce sounds during the breeding season, and their behaviour can be monitored with hydrophones (Ladich *et al.*, 1992; Torricelli *et al.*, 1990). Their sounds are of low frequency and intensity. Only in large aggregations can their sounds be monitored over larger distances.

3.3. Frogs, toads (anurans) and reptiles

Advertisement calls vary much less in anurans (Fig. 1E, F) than *e.g.* in birds (Gerhardt & Huber, 2002), which alleviates automated detection and species assignment of anuran calls (Brandes *et al.*, 2006). In reptiles, crocodilians utter a variety of communication sounds (Vergne *et al.*, 2009) and gekkos too have evolved a vocal repertoire from simple chirps to complex sequences they all use in social behaviour (Brillet & Paillette, 1991; Marcellini, 1974). However, signal characteristics in ectotherms change with ambient temperature (Kuhn & Schneider, 1984; Márquez & Bosch, 1995). This requires recording of soil, water, and air temperature (and relative humidity) for every sound file (Márquez *et al.*, 2008).

3.4. Birds

Birds are acoustically most conspicuous and are regularly being monitored acoustically especially in habitats with low visibility (*e.g.* Bart, 2005; Frommolt *et al.*, 2008b; Haselmayer & Quinn, 2000). The comparatively high song variability (Fig. 2A) within and between individuals makes species identification challenging for observers, and even more so for automated systems (Bardeli *et al.* 2008, Tanttu & Turunen, 2008). Birds such as the nightingale can hold vast and changing song repertoires (Todt & Hultsch, 1996). Nocturnal monitoring of birds along migratory routes, with arrays of directional microphones aimed at the sky (*e.g.* Dierschke, 1989; Evans & Mellinger, 1999; Graber, 1968; Schrama *et al.*, 2008) allows for the challenge of the identification of flight calls, the best possible tool to study such migrations.

In some cases, vocalizations do not only carry information at the species and the geographic, but also the individual level, which allows individual recognition of calling animals (Galeotti & Pavan, 1991; Laiolo *et al.*, 2007).

The use of playbacks to elicit responses of secretive birds has also been developed as a valid census technique (Conway & Gibbs, 2005; McGregor, 1992). Especially in North America, there are several large-scale bird monitoring schemes running (see review in Bart, 2005).

3.5. Terrestrial Mammals

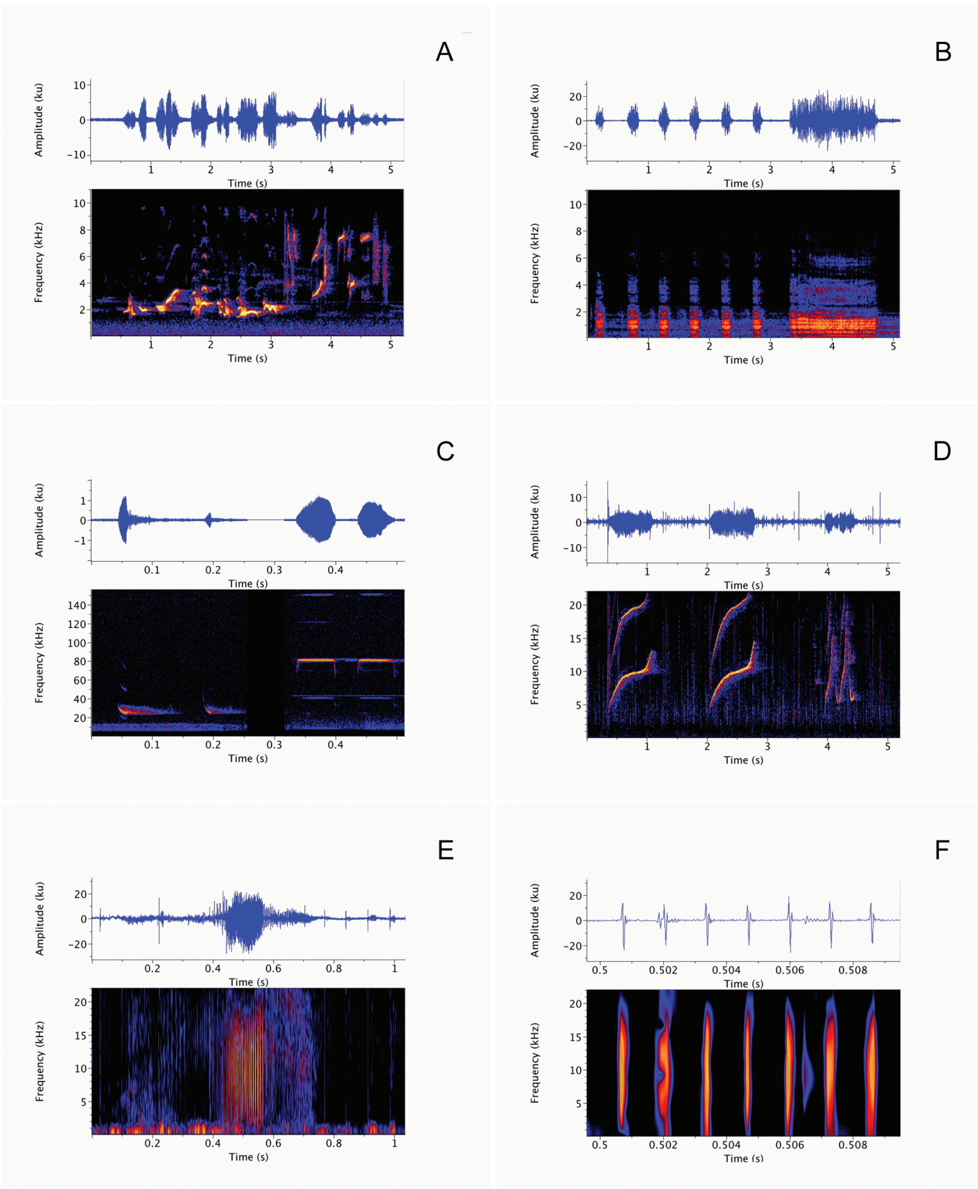
Many mammals extensively use acoustic communication. Individual learning, experience and social contexts condition the development of communication and determine the vocal expression, which overall becomes much more variable than in taxa which show simpler behaviour (Vannoni & McElligott, 2007). But simpler vocalisations like breeding sounds can be monitored to map their presence. Red Deer (*Cervus elaphus*) calls (Fig. 2B) have been extensively recorded eventually resulting in population estimates (Favaretto *et al.,* 2006). Similar studies are made with wolves (*Canis lupus lupus*) by using recordings of their natural call and playback stimulations (Fuller & Sampson, 1988; Gaines *et al.*, 1995; Wilson & Delahay, 2001).

3.6. Bats

Bats do orient, navigate to food sources and roosts, and hunt for prey at night with ultrasound (Fig. 2C). Their mode of orientation was termed 'echolocation' by Griffin (1958). It allows to study bat distribution and behaviour, and has potential for species identification (Ahlén, 1981; Fenton & Bell, 1981). However, this is severely complicated, as sonar calls serve an auto-communicative function and only have limited species or individual specificity. Nevertheless, different technologies are available to monitor and record the inaudible ultrasound (Parsons & Obrist, 2004) (see Technologies section) and recently promising approaches to automated recognition emerge (Jennings *et al.*, 2008, Obrist *et al.,* 2008; Parsons & Jones, 2000; Russo & Jones, 2002; Skowronski & Harris, 2006).

3.7. Marine mammals

The high speed of sound (~1484 m/sec, varying with temperature and depth) and the low attenuation in water favour acoustic orientation and communication in the aquatic environment. As sight is often limited to a few meters distance in water and cannot be used in dark oceanic depths, acoustic communication is the dominant channel of communication in cetaceans. Their signals range spectrally from the very low frequencies of the large baleen whales to the ultrasonic clicks of the echolocating dolphins (Figs. 2 D-F). Their ultrashort biosonar signals (30 to 300 μsec) reach peak source levels of 230 dB re 1μPa/1m and range from 70 kHz to more than 150 kHz (Johnson *et al.* 2004), while social communication usually happens at lower frequencies but still impressive intensities. The distance of whale detection varies widely, depending on signal characteristics and environmental constraints as well as background noise, most of which is caused by man. But during their deep dives up to one hour long, sound is the most efficient way to verify their presence at distances of kilometres. Species with known sounds can be mapped and their movement and behaviour tracked. Techniques to detect and record marine mammals are presented in the Technologies section.



4. Technologies

Apart from a keen human ear, a typical equipment to study animal sounds starts with a microphone (or hydrophone) and a recording device. Progressively more specialized material like directional microphones or parabolas may come into use. For ultrasound generated by many insects, bats and marine mammals, ‘bat detectors’ and specialized equipment for the recording of ultrasounds are needed. Finally, recordings require hard- and software for replay, visualisation, and analysis of the signals. The following section tracks the technical workflow in bioacoustics research: sound pick-up, recording, storing, and analysis.

4.1. Microphones

Microphones contain a mechanically transducing element whose vibrations truthfully convert sound waves into an electrical signal. Different kinds of transducers all generate electrical signal, using electrodynamic, piezoelectric or capacitance and electrostatic effects. The electric representation of the acoustic signal can then be amplified, recorded, visualized, and further analysed or converted back to sound.

In *dynamic microphones*, an electromechanical element generates a current by electromagnetic induction when moved. Such microphones are robust, reliable and do not require external powering, but they have limited sensitivity, making them most useful in loud environments or at close range.

Piezoelectric transducers generate a voltage when stimulated by sound waves. They are used in Hydrophones (see below) and as contact microphones in musical instruments. These devices, historically used *e.g.* in low-cost bat detectors, are very sensitive at their resonant frequency but have variable response at other frequencies (Pye, 1992). To alleviate this, some bat detectors use two different transducers (*e.g.* BatBox III, Stag Electronics, Steyning, UK). A variable response remains and most detectors using these transducers offer only a limited signal output (Heterodyne; see http://www.bioacoustics.myspecies.info) making them unsuitable for spectral analysis. But ruggedness and price make them practical for some type of fieldwork.

Capacitance or *condenser microphones* are more generally suited and most widespread (Pye, 1993). Movement of the diaphragm in the microphone changes capacitance in the pre-charged condenser. Capacitance change is converted to voltage. Two primary types exist: Solid-dielectric and electret microphones.

Fig. 2 (opposite page). Amplitude and spectrographic displays of acoustic signals of birds, and mammals. A. Song of a Blackbird (*Turdus merula*); B. Call of a Red Deer (*Cervus elaphus*); C. Echolocation calls of bats; (left) from a Serotine Bat (*Eptesicus serotinus*), and (right) from a Greater Horseshoe Bat (*Rhinolophus ferrumequinum*); D. Whistle of a Bottlenose Dolphin (*Tursiops truncatus*); E. Buzz of *T. truncatus* in the audible range; F. Series of clicks in a buzz of *T. truncatus*. Note the vastly different time and frequency scales and dB ranges not comparable between subplots! Spectrograms were generated from recordings of the authors.

Solid-dielectric microphones have to be powered, *e.g.* with voltage supplied from the plug (PIP - Power In Plug in consumer products), over the signal cables (*e.g.* 48V phantom powering in professional recorders) or by an internal battery. Such microphones have quite a flat frequency response. They require higher supply-voltages to and are used as laboratory microphones and advanced bat detectors (see http://www.bioacoustics.myspecies.info). Their membranes are mechanically delicate and sensitive to changes in humidity, which can introduce noise into recordings, particularly in humid environments.

In contrast, diaphragms of *electret microphones* are electrically pre-charged, allowing for low power requirements in operation. They are relatively cheap, rugged, very small, and omni-directionally sensitive. Recent products are sensitive up to high ultrasound frequencies. The most recently developed Micro-Electrical-Mechanical System (MEMS) microphones have their pressure-sensitive diaphragm etched directly into a silicon chip with similar fabrication technologies used to make semiconductor devices

In *hydrophones*, the membrane is replaced by a piezoelectric element that produces an electric current when compressed by sound waves propagating under water. Single transducer hydrophones are omni-directional and typically cover a wide range of frequencies, from a few Hz to more than 100 kHz. In the marine environment, more complex array systems are often used to increase directionality and sensitivity. Hydrophones, or arrays of such, are either used in stationary setups to monitor selected areas, or slowly towed over larger regions. Autonomous systems pack hydrophones, amplifiers and a radio transmitter into a floating buoy (sonobuoy) and transmit data to a remote receiver. Packaged with a recorder in a pressure resistant container and deployed on the sea bottom to be retrieved later, underwater sounds can be recorded for a predetermined period. Appropriately sized, such packages can even be attached with suction caps (D-TAG) to an animal, to study its sounds concurrently with its diving profile (speed, depth, orientation), and the sounds it receives (Johnson & Tyack, 2003).

Directional microphones emphasize sounds coming from one direction and a single source, such as an individual singing bird, attenuating ambient sounds. A similar effect can be achieved by parabolas, which reflect sound waves coming from frontal, on-axis directions onto an omni-directional microphone positioned at their focus point. Gain and directionality increase with the ratio of the parabola's diameter to the sound's wavelength. Significant directionality is achieved only for wavelengths shorter than the diameter of the parabola (*e.g.* above 560 Hz with 60 cm Ø). Ultra-directional microphones (*shotgun microphones*) usually are cardioid condenser microphones fitted in a tube, which cancels off-axis signals. These microphones have a flat frequency response, but they are generally less sensitive than parabolic microphones, but rather resistant to wind and handling noise.

Pairs of microphones can be combined to produce *stereophonic recordings*, originally developed to transmit an impression of the spatial arrangement of sound sources. Such recordings can also be processed to emphasize certain sound sources, using software tools for 'source separation'. Stereophonic recording is mostly used to record 'soundscapes', but can also be used for biodiversity monitoring as they convey information on the position of sound sources.

4.2. Digital recording

Quality

In the following description, we refer to recorder devices storing sound files in .wav format initially developed by Microsoft but now in use across all operating platforms (Rumsey & McCormick, 2006). We will not consider consumer electronic products allowing sound recording, such as Camcorders and cell phones. These products use a compressed format for storage (such as mp3 – see Rumsey & McCormick, 2006) that dramatically affects the spectral and temporal composition of the signal. This format is therefore inappropriate for detailed bioacoustic studies even if it could be used for some survey or monitoring work. Appropriate digital recorders reproduce signals with great accuracy, low noise, flat frequency response, and no speed variation. All digital recording devices sample sound with an analogue to digital (A/D)-converter and store the numeric values but not the actual voltage of the signal, on the device. Their usable frequency range is defined by half the sampling rate and the bit depth of the converter, roughly 6 dB per bit, defines the dynamic range. Thus, a 16-bit 44.1 kHz A/D-converter resolves 22.05 kHz with dynamics of 96 dB. High quality digital recording devices should then have an A/D-rate at least twice the highest frequency to be recorded and provide a digital output for lossless transfer.

Recorders

Digital music players and recorders nowadays have become devices of choice to record sound, including slowed down ultrasound. Some models can sample at up to 192 kHz, and some record on up to four channels (see below for ultrasound recording). Most are lightweight and inexpensive, feature large storage capacities and record at high fidelity, if compression algorithms can be switched off. Data are stored on an internal hard disk or on digital CompactFlash (CF), Secure Digital (SD) or SD High Capacity (SDHC) memory cards, all similar to random access memory (RAM) in computers, but with much higher portability.

Recording directly to computer hard disk is well established since the 1980s. Data acquisition boards easily allow for sample rates up to several MHz, enabling direct recording of ultrasound, and affordable hard drives in the Terabyte range can hold weeks of recordings. Laptop computers with large storage capacities now constitute convenient tools to record and visualize sounds directly in the field. They allow a wide choice of sound inputs, sampling rates, and recording channels. Computers also offer the possibility to schedule recordings, allow wide file naming and meta-tagging (timestamp, location, GPS position, ...). Eventually they can be set up to stream sound over wired or wireless networks making remote recording possible. Unfortunately, their internal batteries empty quickly and ask for alternative powering. Furthermore, the internal sound ports of laptop computers are of moderate quality and do not exceed 48 kHz sampling rate. To push quality and increase bandwidth, an external sound input device must be connected over USB, FireWire, or PCMCIA, additionally draining energy. Emerging generations of subnotebooks, small tablet PCs and ever-smarter mobile phones with included GPS will further boost the interest in computer based field recording. A few suppliers of digital recorders, data acquisition hardware, considerations on power requirements and further information resources are listed on http://www.bioacoustics.myspecies.info.

Ultrasound recording

The output of a bat detector allows the recording and permanent storage of nocturnal bat activity. Digital time-expansion bat detectors equipped with a few Megabytes of RAM may be used to autonomously record slowed down chunks of discontinuous recordings to event recorders. However, until very recently, only limited information of a survey could be stored. A time expansion detector can save short recordings to a voice recorder, revealing species-specific information, but hiding total activity information due to the long storage times of typically tenfold the recording duration.

Alternatively, a heterodyning detector, combined with a talking clock records events on a sound-activated tape recorder. This is not suited to inform about species, but nicely keeps track of total activity at a site, *e.g.* as the number of passes per hour (Fenton, 1970). Tapes from such monitoring boxes must be analysed meticulously by listening to them, including their ultrasonic spurious components (*e.g.* rain, insects). Different listeners may interpret events differently, making reproducible species identification difficult.

The Anabat system has become increasingly popular in some regions, but it is harshly debated in others (Barclay, 1999; O'Farrell *et al.*, 1999). It only records a zero-crossing representation of the original signal, which is not sufficient to properly reflect the acoustic variance exhibited in many bat faunas, but it allows for long-term deployment and autonomous signal activation.

Very recently handheld digital storage bat detectors and loggers emerge, which digitally record ultrasound at high sampling rates and bit depths to large enough media, thus permitting full night monitoring of bat activity (for products see http://www.bioacoustics.myspecies.info). Despite their considerable price, combined with automated analyzing and species identification software, such devices promise to become standards and tools of choice for future acoustic bat monitoring. They give not only accurate timing of activity; they also remove human bias from qualitative audiotape analysis, because they allow immediate full spectral analysis of the recorded events.

Automated recording systems (ARS)

Acoustic surveys by human observers are best established in birds. It is an effective method, particularly for the detection of secretive species (Bart, 2005; Conway & Gibbs, 2005). However, increasing interest in long term acoustic monitoring of natural habitats has driven the development of Automatic Recording Systems (ARS), which become increasingly popular and cost-effective

(Brandes, 2008; Hobson *et al.*, 2002; Rempel *et al.,* 2005). Autonomous recording devices could reduce person-hours spent in the field, and lead to a major breakthrough in acoustic monitoring of a wide variety of species, particularly in combination with species recognition algorithms (Frommolt *et al.*, 2008a) and expert listeners.

Most ARS consist of stand-alone processing and storage units, scaling from a simple recorder connected to a timer, to a low-power computer that allows more complex tasks such as scheduled recording or feature triggered on-event recording (*e.g.* amplitude and/or spectral trigger, external sensors). However, energy requirements and storage capacity are still critical delimiters for longer operations.

As with observer based monitoring programs, the design of automated recordings has to be thoroughly planned. Habitat type (transmission conditions), abundance and detectability of target species, as well as the sensitivity and the area covered by an ARS define the number of systems to be deployed and the recording scheme (*e.g.* automated or timed recordings, number of minutes per hour, ...). In temperate regions, anuran populations have a typical aggregate pattern around water resources (Gerhardt & Huber, 2002), thus it is often easy to cover the whole population with one or a few recording sites. Anurans living along rivers or in tropical forests, mammals, birds or even most of the insects have populations more dispersed, which allows only to sample a part of the whole population. Some examples of monitoring programs and equipment are given on http://www.bioacoustics.myspecies.info.

Digital recordings, particularly of ultrasound, quickly expand to vast data quantities. However, they can be copied and archived like any digital data to compact disk (CD, up to 700 MB) or digital versatile disk (DVD, up to 5 GB). But that amount of data is quickly sampled in a few nights with the aforementioned loggers, thus the backing up of Terabytes of sound recordings is presently only feasible to more and more affordable hard disk duplicates. The advent of new recordable media in the multi GB range (*e.g.* blue-ray) will eventually alleviate this archiving problem in the near future.

5. Sound repositories

A strict documentation of recordings is a prerequisite for scientific work with sound. It becomes most evident in species rich groups like insects: explicit meta-data have to be attached to a recording, and in case of poorly known faunas, the collection of voucher specimens is necessary. Alternatively, photographs and/or blood or tissue samples should be collected. Sound databases should preferentially contain signals collected from animals in their natural environment, but reliable association of song and well-curate voucher specimen often requires recording of captured individuals, under controlled conditions. Storage and administration of recordings requires a well-structured database, eventually referenced to voucher specimens. To facilitate search, each acoustic file should refer to a metadata set containing species name and all recording parameters, locality and temperature and ideally be annotated with signal parameters (*e.g.* carrier frequency) preferably extracted by automated algorithms.

Carefully curate sound collections are the pre-requisite for reliable identification of animal calls. Traditionally, so-called phonotheks, or Sound Libraries, established huge repositories initially based on analogue tape recordings (*e.g.* Tierstimmenarchiv Berlin, British Library Sound Archive's wildlife collection or the Macaulay Library of Sounds).

Over time, bioacoustic collections suffer from degradation of the recording media (tapes), and the obsolescence of suitable playback equipment. Digitalisation is time-consuming, but a solution that can keep recordings alive and usable, if the data are stored in an exchangeable standard format (AIFF, WAV) and are regularly transcribed within the lifecycle of one media type (20-40 years) to a more recent one. Most importantly, a presentation on the Internet today is the method of choice to enable access to a wide community of users. The International Bioacoustics Council (http://www.ibac.info/index.html) provides a comprehensive list of links to all major sound archives. A portal providing federated access to distinct sound archives, with a unified query tool for sound archives would be highly desirable, and could eventually be implemented through the Global Biodiversity Information Facility (http://www.gbif.org/).

6. Sound display and analysis

Today, most bioacoustic signals are digitally recorded (see Technologies section). This allows easy data filing and retrieval for signal analysis, to reveal the species-specific acoustic parameters for the recorded species. Digital recordings can be recorded, played and edited by standard software contained within Windows, Linux, and Apple operating systems. However, additional software packages are needed to visualise songs and quantify relevant parameters such as temporal structure and frequency composition (see Figs 1 & 2). Software ranges from simple freeware to very powerful open source or commercial products, some of which allow implementation of automated detection and recognition algorithms (see http://www.bioacoustics.myspecies.info).

The simplest graphical display of a signal is an oscillogram, revealing temporal changes of sound pressure, usually transformed into voltage amplitude by a microphone (top in Figs 1 & 2A-F). Further information is revealed by the frequency composition of a signal at any given moment, generally based on a windowed Fast Fourier Transform (FFT). Most meaningful and widely used is the display of a series of spectra, computed on consecutive and generally overlapping segments of a signal, called a spectrogram (see http://www.bioacoustics.myspecies.info). This shows the evolution of the frequency structure (y-axis) of a signal over time (x-axis), where intensity (z-axis) is coded as brightness or on a colour palette (bottom in Figs 1 & 2A-F).

A spectrogram can reveal sound features humans cannot perceive, such as fast frequency or amplitude modulations, or frequency components outside the human hearing range, *e.g.* infrasounds emitted by some large whales or by elephants (Garstang, 2004), as well as ultrasounds emitted by echolocating dolphins or bats. A real-time spectrograph can continuously display the results of a spectral analysis of incoming sounds, even in the field while recording. Spectrograms can be used to measure characteristics of a signal either manually or with automated algorithms readily offered by some programs. Nevertheless, a detailed study of the settings and rules of the software and a basic experience in bioacoustics is required to achieve reproducible and meaningful results (see Appendix A & B in Charif *et al.*, 2009; Cortopassi, 2006). Examples of such tools are given below. The Raven-Lite software is even available as a plug-in for web-browsers, allowing web-based, immediate display and analysis of the vast collection of field recordings available at the Macaulay Sound Library (http://www.macaulaylibrary.org).

Three methods make ultrasound audible for humans and allow real-time analysis of bat echolocation calls or high-pitched insect sounds in the field: heterodyne frequency shifting, frequency division, and time expansion. Only the latter conserves full signal content. The most advanced bat detectors incorporate all these systems to make ultrasounds audible and recordable (see Parsons & Obrist, 2004 and http://www.bioacoustics.myspecies.info). In case of continuous wideband recordings, just slowing down the recording makes the ultrasounds audible.

7. Analysis software

Software for sound editing and generic sound analysis can be found on the Internet, either freeware or open source (*e.g.* AUDACITY), or commercial, *e.g.* ADOBE AUDITION (commercial, formerly CoolEdit). Very few programs are dedicated to bioacoustic use and in the following we alphabetically list and summarize the functionality of the more established ones that are actively developed and supported. Other valuable software dedicated to bioacoustics are *e.g.* ISHMAEL, PRAAT, and SYRINX.

7.1. Avisoft

Avisoft-SASLab Pro is Windows software developed by Raimund Specht (Avisoft Bioacoustics, Berlin, Germany - http://www.avisoft.com). Avisoft is a versatile sound analysis, editing, classification and synthesis tool made portable by a dongle copy protection system. It provides analyses including amplitude envelope, FFT, filters, labels, LPC, cepstral analysis, auto- and cross-correlation. Time and frequency measurement can be taken automatically through a sound element detection process. Syllable automated classification can be run by means of a template cross-correlation algorithm and a dedicated pulse train analysis supports the investigation of temporal patterns of both simple pulse trains and burst series. Sounds can be generated with a user-friendly graphical interface. Avisoft includes a tool to manage georeferenced wav-files recorded with a digital field recorder using GPS track log data. Avisoft-RECORDER is a separate application interface for multichannel triggering of hard disk recording systems for *e.g.* long-term monitoring and acoustic event recording.

7.2. BatSound

Batsound is Windows software (Pettersson Electronics, Sweden - http://www.batsound.com/psonan.html) enables the user to digitize a signal using the computer's built-in sound card, and view its temporal and spectral content using Fourier or zero-crossing analysis. In conjunction with high-speed A/D hardware, the software is also capable of digitizing sounds at 300-500 kHz making real-time recording of unaltered signals possible on laptop computers in the field.

7.3. Raven

Raven is commercial full-featured sound analysis software running on Mac OS X, Linux and Windows. It allows recording, processing, analysing and viewing files in a great variety of ways. It sports automatic measurements of signal characteristics, configurable detectors and correlators and allows batch processing of extensive data sets. The full version can be tested (time-limited) and a less powerful free version is available. The Software supersedes the earlier program Canary, which was only running on Mac OS. The software is actively developed at the Cornell Laboratory of Ornithology (Cornell University, Ithaca, NY, USA) and available from http://www.birds.cornell.edu/raven.

7.4. SeaPro

SeaPro (Windows, available in a free version) was developed at CIBRA for bioacoustic research to provide real-time sound analysis capabilities and continuous recording to hard disk (http://www.unipv.it/cibra/res_software_uk.html). For marine mammals ship-based surveys it allows continuous real-time display and recording of multiple channels 24h/day, in 15, 30, or 60 minutes long geo- and time-referenced wav files. For browsing wav files collections, it allows high-speed display, and playback at lower or higher speed. It can also be programmed to do scheduled recordings or to record only when sound energy exceeds a given threshold in a user defined frequency range.

7.5. Seewave

Seewave (Sueur *et al*., 2008) is an extension of R, an open source environment (Windows, MacOS, Linux, FreeBSD) for data manipulation, calculation, statistical computing and graphic display. Seewave is command-line driven allowing users to adapt embedded functions to their own needs, to write their personal functions for new analysis or to develop scripts for batch processing. Sounds are edited as oscillogram or envelope in single or multi-framed windows. Signal and silence durations can be automatically measured. In the frequency domain, several statistical descriptive parameters (dominant peak, quality factor, entropy, spectral flatness, …) can be extracted. The fundamental frequency of harmonic series is detected by the autocorrelation or cepstral method, while the instantaneous frequency is obtained by the zero-crossing method or Hilbert transform. Seewave provides 2D and 3D spectrograms. Cross-correlations, surface computation and coherence between two samples can be computed. Any mathematical operations between different sounds can be achieved. Amplitude filters, frequency filters, linear frequency shifts are also available.

7.6. Song Scope

Song Scope is another software available to automatically detect animal songs in large series of field recordings. This is a package for Windows, Mac and Linux platform developed by WildLife Acoustics Inc. (http://www.wildlifeacoustics.org). The program uses complex digital signal processing algorithms that are based on Hidden Markov Models (HMM). The Song Scope´s models or recognizers are built from training data of the species vocalizations (annotations) and after setting several parameters it is capable to accurately identify species in field recordings. The algorithm considers the spectral and temporal features of individual syllables and how syllables are organized into more complex songs. To identify sounds, Song Scope requires training data of every target species, *e.g.* from high quality recordings from sound libraries. The software allows extensive control over temporal and spectral settings, which reversely requires some knowledge and learning of the settings.

7.7. X-Bat

The software X-Bat was developed at the Bioacoustics Research Program of the Cornell Laboratory of Ornithology (Cornell University, Ithaca, NY, USA - http://xbat.org). This software is a free extensible sound analysis application but it requires the commercial MatLab platform. X-Bat runs under Windows, Linux and Mac OS X and is especially useful to work with large-scale sound data where it still responds quickly and efficiently. X-Bat contains highly adjustable ‘Data Template’ detectors (spectrogram cross-correlator) for the efficient detection of signal types in large data sets. Furthermore, X-Bat allows to include new functions for specific tasks by scripts programmed in the MatLab language.

8. Bioacoustic inventories

The concept of biodiversity encompasses several levels of biotic variation - from alleles to landscapes - and has thus lead to a plethora of assessment methods (Purvis & Hector, 2000). Species richness is an important aspect of biodiversity (Magurran, 2004) and bioacoustics offers an access to measure it (Fig. 3). Compared to established collecting methods like catching and trapping, visual or auditory contact is probably the easiest way to substantiate a species’ occurrence and estimate biodiversity.

An acoustic inventory may cover a majority of species in some taxonomic groups (birds, bats, Orthoptera), but it will still be an incomplete estimation of total biodiversity, as it is limited to a set of acoustically conspicuous species.

The simplest acoustic surveys consist of write-downs of audible sounds heard by human ear. Scientific scrutiny requires a proof of observation, a sound recording, which can be subject to spectrographic viewing (Diwakar *et al.*, 2007) or sound analysis (Riede, 1993; 1997) to support auditory identification. Recordings can ease and fasten the assessment process, enable double-checks of species identification, and thereby reduce inter-observer variance.

Where experts are scarce or species unknown, parataxonomic classification of morphospecies or Recognizable Taxonomic Units (RTU), could be applicable, an approach undertaken in Rapid Biodiversity Assessment (RBA) programs (Basset *et al.*, 2000; Oliver & Beattie, 1993).

8.1. Survey Methods

Point-counts or acoustic identifications along transects are simple methods used mainly for the assessment of amphibian or bird populations. This approach seems efficient, but is limited by the brief observation time, the long expert training, and a potential observer effect (hearing threshold and recognition processes). Recent Automated Digital Recording Systems (ADRS) allow acoustic surveys for extended time periods (Acevedo & Villanueva-Rivera, 2006), gathering data at a fraction of the cost for field observers.

8.2. Automated identification

To further standardise, and gain expert independence, computer-aided call classification and species identification tools have been developed for several taxonomic groups. Different detection and classification methods have been tested on bats, marine mammals, birds, amphibians, and insects (Brandes *et al.,* 2006; Chen & Maher, 2006; Obrist *et al.*, 2004; Parsons & Jones, 2000). Most of these approaches reach respectable recognition rates up to 90%, but rarely cover all species to be expected. Despite the need for extensive preliminary studies to establish templates for recognition, standardized self-running approaches are very attractive for monitoring target groups such as marine mammals or bats, but they remain challenging when investigating taxon-rich communities.

8.3. Rapid Acoustic Survey, ambience or soundscape recording

A fairly new acoustic approach goes beyond the species level, measuring bioacoustic diversity for the entire community. A Rapid Acoustic Survey (RAS) analyses the whole soundscape produced by the local animal community and gets a global measure of it (Sueur *et al.*, 2008). As such, RAS goes beyond a RBA by trying to identify neither species nor phonotypes, but rather assess both temporal and frequency heterogeneity − or entropy − of the composite soundscape produced by the acoustic community. Because of competition for sound niches in time and frequency, a more heterogeneous spectrum and amplitude envelope can be expected from a higher biodiversity of singing animals. Signal entropy was quantified by a Shannon-like formula, producing a surrogate for α biodiversity at a certain locality and for a certain time (the algorithm is available within the R acoustic package “seewave”: Sueur *et al.*, 2008). Beta diversity can then be calculated from the acoustic dissimilarity between pairs of recordings, which exhibit envelope and spectral surface differences. So far RAS has only been tested on simulated communities and on the dawn and dusk soundscapes of two coastal forests in Tanzania. All simulations and tests were promising and revealed significant acoustic differences between the two African forests, with a lower α index for the forest disturbed by logging. The method has now to be tested in different habitats − temperate and tropical, terrestrial and aquatic − on broader time and spatial scales. Results should also to be confronted with classical surveys. RAS will not replace classical surveys based on a knowledge in taxonomy but will rather help in getting a fast estimation of local diversity, and rapid results can be obtained by untrained personel.

Even if they do not solve the classical sampling problems encountered by other biodiversity surveys and even if they are sensitive to noise, all acoustic methods reported here can be considered as a valuable tool when documenting biodiversity. Automatisation and availability of recording stations will increase in the next years and provide valuable baseline data to identify hotspots of biodiversity. Efficient data processing and linking of stations will allow timely detection of biodiversity declines, which is necessary for pinpointing current alarming threats to biodiversity.

9. Setting up an inventory

The appropriate procedure for bioacoustic recording depends on the purpose and animal group. You can spend a lot of money in high-sensitivity condenser microphones, only to realise that they do not work during rainforest dusk, when you always have the atmosphere saturated with humidity. This will cause hissing in the condenser microphone membranes, unless you use a (even more expensive) heating device. However, much cheaper electret microphones work fine.

At present, no generally accepted standard protocol for bioacoustic monitoring is available, and quite distinct procedures are used depending on the taxonomic group (*e.g.* for South American frogs and insects, see Brandes 2005). The key at the end of this paper should help the novice to select the appropriate bioacoustic technique. However, there are still a variety of pitfalls and major crosscutting issues to be carefully considered during bioacoustic work, some of which will be discussed below. In any case, it is highly recommended to discuss major bioacoustic projects with experienced researchers.

9.1. Detection space

Any acoustic monitoring has to take into consideration the active space of the recording situation, defined as “that distance from the source over which its amplitude remains above the detection threshold of potential receivers” (Brenowitz, 1982). The detection space depends on sender (calling animal), environment (transmission characteristics) and the receiver (microphone, recorder, ...). For an ARS this definition can be extended to the area around the ARS where calls of the target species can be recorded and identified. Detection space determines the number of stations necessary to quantitatively monitor a particular habitat or population, and to compare the data between stations. Although this quantification can be performed empirically with playback tests, it is often not feasible (*e.g.* for species assemblages, or for bats) and setting stations to equal recording levels only certifies comparability of relative activity.

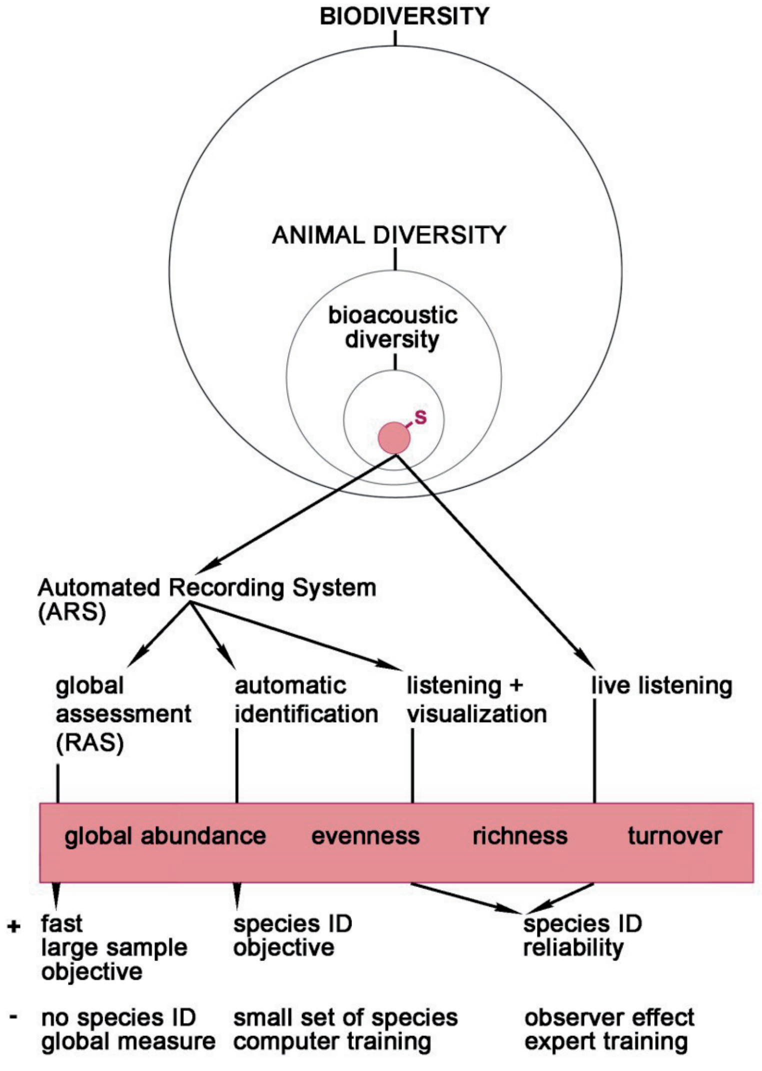


Fig. 3. Scheme of the different acoustic methods currently used to assess biodiversity. Acoustic survey focuses on a selected part of biodiversity. This sample can be directly analysed by the help of expert listeners or by post-recording analyses. All methods try to estimate the main parameters used when measuring biodiversity (global abundance, richness, evenness, and turnover). The main advantages (+) and disadvantages (-) are reported.

9.2. Noise

Noise can have major impacts on both marine and terrestrial ecosystems. Wind and noise coming from human activities (roads, airplanes) also pose a major problem in outdoor recordings. Wind can be attenuated with proper windshields on the microphones, but neither traffic nor competing calling animals (*e.g.* Orthoptera when interested in bats) can be avoided. Self-noise of microphones is another problem. It is normally expressed with an A-weighted or linear dB value. Values range from below 10 dB(A) for very quiet microphones to above 20 dB(A), which is too high for ambience recordings or quiet sounds.

The possible effects of environmental background noise, sound attenuation by multiple products of distance, humidity and frequency, directionality of emitting bats and recording devices, and last but not least Doppler effects, on ultrasound recordings are all comprehensively reviewed by Pye & Langbauer (1998).

9.3. Mechanical sturdiness and damage

Microphones are the most vulnerable parts of any recording chain. Some commercially available units have somewhat weather resistant membranes but it is essential to prevent direct contact between the microphone capsule and water. Possible protective measures are detailed (http://www.bioacoustics.myspecies.info).

Finally, the possibility of wilful human (or animal) destruction always exists and recording devices may have to be accordingly camouflaged, hidden or protected.

9.4. Anti-Aliasing recordings

When digitizing analogue signals, sampling rates must be at least twice the rate of the highest signal expected in the recording. Undersampled signals depict artificial spurious components in the spectrogram display. Thus, a low-pass-filtering adept to the digitizing hardware should be employed to the incoming signal. Most recorders and A/D converter boxes incorporate decent filters, but aliasing occasionally may still appear in spectrograms of very loud components of sounds (see http://www.unipv.it/cibra/res_techtest_uk.html).

9.5. Clipping

Outdoor recordings may contain a sequence of vocalisations calls emitted by a passing animal (*e.g.* flying bat). During a passage by the microphone, the recorded signal's intensity changes. If the intensity of a recorded signal surpasses the upper limit of the recording system (detector, tape, A/D-converter), the signal will be clipped, setting recorded values constantly to the maximum level recordable, thus creating spectral components not present in the original signal. When visually monitoring playbacks such overload signals can easily be identified.

9.6. Doppler effects and more

Depending on speed and frequency of a sender, a resulting Doppler effect can amount to several kHz at the peak energy of *e.g.* echolocation calls in bats, thereby easily surpassing interindividual variability (Obrist, 1995). This can seriously hamper the identification of species, which occur concurrently and show overlap of frequency bands due to Doppler-effects created by different flight speeds or directions (*e.g. Pipistrellus nathusii* and *Pipistrellus kuhli*). Bats hunting concurrently may also interact acoustically, thereby altering their preferred frequency range considerably (Habersetzer, 1981; Obrist, 1995). In such cases, species may be confused unless the track of calls is acoustically and visually verified by an observer on a spectrogram. When recording with a digital system, it is advisable to keep the peak amplitude well below the 0 dB mark on the level display.

10. Key for the selection of bioacoustic procedures

The following key systematises the wide variety of available bioacoustic techniques and purposes. Together with the online material accompanying this manual, it hopefully stimulates biodiversity researchers to enrich inventories with bioacoustic data.

10.1. Recordings for personal reference and later use of bioacoustic keys, or as evidence for occurrence of a certain species.

A wide variety of (cheap) equipment can be used, including automatic recording devices using sound compression. Try to join other naturalists interested in "your" target group, and select similar equipment and protocols. In any case, annotate and archive your recordings as described below and share your data and make them available through web2.0 sites (http://observado.org/sound/index).

10.2. Recordings for scientific use, such as detailed song analysis or for taxonomic description.

10.2.1. Target taxon generates audible sound.

- Target taxon vocalises and can be recorded **in captivity**.

  More detailed and sophisticated measurements and recordings can be made in captivity, using soundproof chambers, sound level recorders (db-meters) or laser vibrometer. Experienced bioacousticians usually employ these techniques, but taxonomists also use recording captured individuals, mainly to obtain a voucher specimen. For insects in particular one should always try to obtain voucher specimens from recordings made in captivity. Recordings and specimen should be cross-referenced by adequate labelling and storing in a database; temperature and light conditions must be annotated, together with technical details. Use high sample rates and uncompressed storage formats (*e.g.* wav format). After publication, share your data by depositing recordings in public phonotheks and/or databases.

Voucher specimen should be deposited in a recognised Natural History Museum (this is a must for species descriptions).

- Target taxon recorded **in the field**.

  Many taxa do not sing in captivity, are too rare, endangered, and/or protected by law to be caught, or the investigator studies bioacoustic problems in an otherwise well-known species (mostly birds and mammals, but also European insects). Select field-recording equipment adapted to animal group, biotope and budget (see section field recording). Annotate recordings. Use high sample rates and uncompressed storage formats (*e.g.* wav format). After publication, share your data by depositing recordings in public phonotheks and/or databases.

10.2.2. Target taxon generates ultrasound components.

Species specificity of signals is only guaranteed in open flight situations! Signals emitted in captivity cannot be compared to outdoor recordings and are mostly inappropriate for species identification!

Be aware of the observer effect: bats are curious and sensible and obtrusive observer presence could bias recordings!

- Bat **presence** to be determined (without accurate species identification).

  Heterodyning or frequency division bat detector is sufficient.

- **Species** to be determined.

Time expansion detector and digital recorder required. Keep in mind that for the same recording duration digitized ultrasound takes roughly tenfold the data space of audio recordings.

Species identification at a roost.

Stationary recording of the signals at 10-20 meters distance from the roost in the flight path of the emerging bats is required to register standardized orientation calls. Different species leave the roost at different times, thus prolonged recording until about 2 hrs past sunset is recommended.

Species identification at a distinct foraging site, streetlight, pond, ...

Stationary recording of the signals is recommended. Switching the recording position in intervals of ≥ 30 min may detect more species using the site[1].

Species habitat use, presence in a landscape.

Slowly walk a predefined transect: avoid walking on gravel (ultrasound noise!). Dim down your headlight[1].

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[1] At least one repetition of the survey is necessary, preferably at different daytimes and seasons to account for different detectability of species. Log date, position, habitat type, type of recording equipment (digitization parameters), as well as wind and temperature conditions. Store the data for future reference and share it with the scientific community by

Stationary recording at several points: record for at least 30 min at each predefined site. If possible use several detectors concurrently[1].

11. Checklist to successful recording

Before you start

Weather

Check weather forecast and avoid rainy and windy days (or use wind protection).

High humidity environments (*e.g.*, tropical rainforests, ponds in cold nights, etc.) may damage your recordings. Care for a replacement microphone.

General equipment

Check your equipment (batteries, leads, connectors, ...).

Always take spare batteries with you.

Know your equipment perfectly: you should be able to run a recording without seeing the buttons of the recorder.

Carry some silica gel in airtight bags to dry microphones when not in use.

Use headphones to monitor the field recording recorder (quality, level, background, ...), and you will be able to correct in advance some problems and improve your recordings.

Calibrate the recording equipment before and after recording sessions with a calibrator device. Together with an accurate measurement of recording distance this is essential to calculate intensity parameters from the recordings. Use identical ARS' and calibrate them to allow later comparison between recording stations.

Microphones

Close sound source expected

Use an omnidirectional or cardioid microphone with a frequency response as flat as possible.

Distant sound source

Use a directional microphone (shotgun microphones or a parabola) to record focused on distant individuals with the best signal to noise ratio.

Consider two-channel recording to record the acoustic context and the focus animal. Different callers at different positions can easier be distinguished in binaural recordings.

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depositing recordings in public phonotheks and/or databases. (For further details see Brigham *et al.*, 2004; Kunz, 1988).

Out in the field: noise and site selection

If possible, choose an isolated site, away from all sources of anthropogenic (road, airport, train, city) or natural noise (stream, waterfall), including other acoustically active species not targeted.

Place hydrophones where the water is still. Avoid running water. At sea, suspend the hydrophone with progressive sub-surface floaters to allow it to sink and stay stable at the desired depth, unaffected by surface movements (boat).

Keeping track: the protocol

Have a fieldwork paper book to note as much information as possible you would not remember the day after.

Describe the habitat and more specifically the close environment around the source.

Keep notes of the equipment and take photographs of it and of microphone positions.

Record the local weather parameters (air temperature in the shape, air temperature at the insect position, relative humidity, wind force, cloud cover).

At the beginning and at the end of a recording session, also record verbally all the relevant information you wrote in your field journal: date (yes, including year!), time, localisation (if possible GPS coordinates), weather (especially temperature for amphibians or insects), habitat, background noise, recording equipment, recording author, ...

Give a field identification number to the specimen recorded.

Observer behaviour

Move as little as possible. You may even sit down and let your target animals approach.

Be patient. Before changing your recording site, wait at least 20 minutes. Insects start to sing again!

Make as many comments as possible before or after and not during the recording.

During the session, only record verbally *e.g.* subject changes, which will be useful for later analysis. This should also be done every time an ARS is set in the field or serviced.

Recording

Keep similar distances to subjects: one meter is usually a good distance for insects.

Direct the microphone away from possible noise sources.

Avoid the recording of sound reflected from surfaces (ground, water) by pointing the microphone at the subject in parallel to that surface.

Avoid overloaded recordings: don't put the recording level too high (recorder clipping risk) and don't put the microphone too close to the source (microphone clipping risk).

Regularly check the input sound level during recording, and learn how to detect a clipped signal by listening to the headphones.

High bit-depth digital recorders give good recordings even with reduced recording levels.

Use a sampling rate reasonably higher than strictly needed, to preserve the wider spectral context in which a vocalization occurs.

Housekeeping

Transfer all your data to a laptop computer and/or an external hard disk. Be sure that no digital re-sampling occurs when transferring the original files. Try to transfer daily to avoid confusion between files.

Organize and name your files and folders very clearly.

Lock the recorded files in order to preserve the creation date (some sound editors will modify the metadata of the file as soon as you open them).

Generate a database (from a table to a true database) describing your recordings.

Keep note of the recording settings (number of channels, bits and format, sampling rate); if a file header is corrupted, this helps to recover the file.

Backup your data.

Deposit the recordings in a scientific sound library.

Analysis

Set spectrographic parameters carefully (windowing, overlap, FFT-size). To match slowly or quickly changing sound parameters (*e.g.* whole insect chirps or individual pulses within the chirps) you may need two different settings.

Use those constant time-frequency scales, dB scales and spectrogram size to make comparisons easier. Take note of the settings (*e.g.* screen capture).

Avoid too much filtering or noise reduction except low noisy frequencies (wind etc.)

Take robust temporal and spectral measures (Cortopassi 2006).

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