Studying the biosonar of bats and porpoises using multi receiver arrays

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1 Summary

Echolocation, the main sensory modality of odontocetes and microbats, has mostly been studied using single receivers. Such a recording setup is sufficient to study signal parameters like pulse interval, inter click interval or terminal frequency. However, the usually high frequencies and high directionality of echolocation signals do not allow precise measurements of start frequency, signal intensity and emission directionality using a single receiver. Recording impinging signals with multiple receivers in a defined spatial arrangement allows sampling the emitted signal simultaneously at different angles relative to the acoustic axis. Such arrays have first been used to study emission directionality of stationary animals at a known location relative to the array. Starting in 1990, arrays were used more extensively to first locate an echolocator based on the time of arrival difference of the emitted call at the different receivers. In a second step, the signal parameters of the recorded sounds were then analyzed and measurements of first signal intensity and later directionality of free ranging animals were obtained.

In the course of this doctorate, I first used a two dimensional 16 microphone array to measure the signal intensity and the variation thereof of big brown bats in a flight room. The position of the animals was determined by stereo infrared video recordings. I confirmed that the signal intensity decreased as the bats flew across the flight room towards a landing platform. The intensity reduction however was not constant but showed oscillations of up to 12 dB within a few calls. These oscillations were linked to the wingbeat, indicating an effect of wing movement on the call intensity. In addition, the call emission timing was linked to the wingbeat cycle. Detailed analysis revealed that single calls were emitted during the upstroke. When the bats emitted groups of calls, signal emission started earlier during the wingbeat cycle.

The two dimensional microphone array was then increased in size to four by four meters and adapted for recordings of free ranging bats in the field. Bats were first localized by videogrammetry, later during the doctorate by acoustic localization. Introducing the latter to the department of animal physiology was one of my main contributions during my doctorate. I developed additional algorithms which resulted in the ability to separate signals emitted by multiple individual echolocators and to measure the sonar parameters of each individual.

In addition to bats, I studied the directionality and source levels of porpoise clicks. In a first approach, I used a two dimensional plus shaped 16 hydrophone array to measure the directionality of single clicks emitted by a stationary animal in both the horizontal and vertical dimension separately. The sonar beam was found to be narrower than reported previously and the directionality to some extend dynamic. To exclude individual differences of the two animals used in the two studies, I carried out a further experiment. Clicks by two additional porpoises were recorded with a regularly spaced wall of 15 hydrophones while catching prey. The porpoise’s position at click emission was determined using acoustic localization and the intensity, directionality and direction of signal emission relative to the body movement were studied. The results from these three freely moving animals confirmed the narrow beam in harbor porpoises.

2 Zusammenfassung

Die Echoortung von Zahnwalen und Fledermäusen – ihr vorrangiger Wahrnehmungssinn – wurde meist mit einzelnen Empfängern untersucht. Damit können Signalparameter wie Lautabstand und Endfrequenz untersucht werden. Allerdings können die Anfangsfrequenz, Intensität und Richtcharakteristik dieser hochfrequenten und gerichteten Echoortungslaute mit einzelnen Empfängern nicht genau bestimmt werden. Diese Parameter der Echoortungssignale können jedoch mit mehreren Empfängern aufgezeichnet werden, die sich in einer festgesetzten räumlichen Anordnung befinden und somit das Signal simultan in unterschiedlichen Winkeln zur akustischen Achse messen. Diese sogenannten Arrays wurden ursprünglich genutzt um die Richtcharakteristik der Echoortungssignale von stationären Tieren zu messen, welche sich an einer festgelegten Position aufhielten. Seit 1990 wurden Arrays vermehrt eingesetzt um erst ein echoortendes Tier, basierend auf den akustischen Laufzeitunterschieden, zu lokalisieren. In einem weiteren Schritt wurden dann die Signalparameter analysiert und erst die Intensität, später auch die Richtcharakteristik von sich frei bewegenden Tieren gemessen.

Im Rahmen dieser Doktorarbeit wurde zunächst ein zweidimensionales Array aus 16 Mikrofonen genutzt, um die Intensität und die Intensitätsvariation von Großen Braunen Fledermäusen in einem Flugraum zu vermessen. Die Position der Tiere wurde zugleich über Stereo-Infrarot-Videoaufnahmen bestimmt. Die Aufnahmen bestätigten, dass die Tiere während des Anflugs einer Landeplattform ihre ausgesandten Signale reduzierten. Diese Signalreduktion war jedoch nicht konstant sondern oszillierte im Zuge weniger Laute um bis zu 12 dB. Die Oszillationen ließen sich mit dem Flügelschlag korrelieren, was auf einen ursächlichen Zusammenhang hindeutet. Zusätzlich war der Zeitpunkt der Lautaussendung an den Flügelschlag gekoppelt. Detaillierte Analysen zeigten, dass Einzellaute während des Aufwärtsschlags ausgesandt wurden. Sandten die Fledermäuse dagegen Lautgruppen aus, begannen diese bereits früher im Flügelschlagzyklus.

Das zweidimensionale Mikrofonarray wurde später auf vier mal vier Meter vergrößert und an den Freilandeinsatz angepasst. Die Position der Fledermäuse wurde anfangs anhand von Videogrammetrie und im späteren Verlauf der Doktorarbeit per akustischer Lokalisation bestimmt. Diese Einführung der akustischen Lokalisation in den Tübinger Lehrstuhl für Tierphysiologie war einer der Hauptbeiträge im Zuge dieser Doktorarbeit. Zudem wurden Algorithmen entwickelt, welche es erlauben, die Echoortungssignale mehrerer gleichzeitig rufender Tiere den jeweiligen Einzeltieren zuzuordnen und somit deren individuelle Echoortungsparameter zu bestimmen.

Zusätzlich zu Fledermäusen wurde im Zuge dieser Arbeit auch mit Schweinswalen gearbeitet und von letzteren die Richtcharakteristik und Intensität ihrer Klicklaute untersucht. In einer ersten Herangehensweise wurde ein zweidimensionales, plusförmiges Array aus 16 Hydrophonen benutzt, um die horizontale und vertikale Richtcharakteristik der Einzellaute eines stationären Tieres zu messen. Es zeigte sich, dass die Schallkeule dynamisch war und zudem enger als in bisherigen Publikationen angegeben. Um individuelle Unterschiede zwischen den zwei Tieren der beiden Studien auszuschließen, wurde ein weiteres Experiment durchgeführt: Die Laute zweier weiterer Schweinswale wurden während des Beutefanges mit einer Wand aus 15 regelmäßig angeordneten Hydrophonen aufgezeichnet. Die Position der Schweinswale bei der Lautaussendung wurde durch akustische Lokalisation bestimmt und die Intensität, Richtcharakteristik und Aussenderichtung relativ zur Bewegungsrichtung der Tiere untersucht. Die Ergebnisse dieser Studie bestätigen die enge Schallkeule bei Schweinswalen.

3 Glossary

ADC	analog to digital converter
ASL	apparent source level
BW	beam width
CF	constant frequency
CPU	central processing unit
dB	decibel
DC	direct current
DFTBA	don’t forget to be awesome
DI	Directionality index
FFT	fast Fourier transformation
FM	frequency modulated
GPS	Global Positioning System
ICI	inter click interval, time between call emission to previous call
IR	infrared
MINNA	minimum receiver number array
ODA	over determined array
PI	pulse interval
pp	peak to peak
PSD	Power spectral density
RL	received level
RMS	root mean square
SL	source level
SNR	signal to noise ratio
SPL	sound pressure level
SR	sampling rate
TOAD	time of arrival difference

4 Introduction

4.1 Biosonar of bats and toothed whales

Microbats (Microchiroptera) and toothed whales (Odontoceti) have both evolved the same highly specialized sense: echolocation. High frequency signals are emitted and returning echoes enable the animals to orient themselves in space and detect, localize and classify prey (Au, 1993; Griffin, 1958). The proof for the use of ultrasonic sound for orientation in bats was only made in 1942 by Galambos and Griffin (1942), but had already been suspected by Spallanzani two centuries earlier. The final proof that dolphins use the same sense was published even two decades later in 1961 by Norris (Norris et al., 1961). The use of echolocation in toothed whales had been suspected and investigated a few years earlier, however in all previous experiments vison was not completely excluded (Au, 1993).

4.1.1 Sound production of bats and toothed whales

Bats produce ultrasonic calls with their larynx. These calls are altered by mouth cavity and/or nasal passage and consequently emitted through the nostrils or mouth or in at least one species through both (Seibert et al., submitted). Odontocetes produce short clicks by pushing air past a pair of phonic lips. The signal propagates then rostrally through a fatty melon (composed of fat with different density) and enters the water as a focused sonar beam (Cranford, 2000; Cranford et al., 1996).

4.1.2 Physical properties of the emitted and received sound

Echolocation signals emitted by both taxa in the two media, air and water, are in general prone to the same basic physical effects. However, the magnitude of some of the effects differs drastically.

4.1.2.1 Speed of sound in air and water

One of the fundamental differences between air and water is the speed of sound: In air, sound propagates at 340 m/s whereas in water, it travels with 1500 m/s and thus with more than four times the speed than in air. Moreover, the speed of sound in air depends on pressure, humidity and temperature. Analogously in water, the speed of sound is influenced by pressure, temperature and salinity.

4.1.2.2 Spherical spreading of sound

When a signal, emitted by a source, propagates through any media, the emitted energy spreads over a larger area, hence reducing the intensity with increasing distance. The loss in dB due to this spherical spreading can be computed by 20*log(distance). This leads to sound intensity reduction of 20 dB for every tenfold increase of distance and applies to both air and water.

4.1.2.3 Absorption of sound

In addition to spherical spreading, the emitted signal is affected by the absorption in the medium it travels through. This is energy conversion from motion to heat as the media particles collide with each other. Therefore, higher frequencies are generally attenuated stronger than lower frequencies. Attenuation in air has a strong influence on the echolocation range of bats (Stilz and Schnitzler, 2012). In water, absorption has a comparably smaller effect on the attenuation of echolocation clicks. In air, absorption depends on temperature, humidity, atmospheric pressure and frequency. In water, attenuation depends on salinity, frequency and pressure.

4.1.2.4 Frequency dependent resolution of sound when used for echolocation

Due to physical constraints, low frequencies can only be effectively emitted by relatively large sources. These low frequency signals carry further. However, due to the long wavelength, the lower frequency signals are only reflected by larger objects and hence restrict the animals especially in prey choice to larger prey. The higher the frequency, the smaller the object that can be detected by the echolocator. This allows high frequency emitting species to prey on smaller prey items than low frequency emitting species.

4.1.2.5 Directionality of sound

Directionality is caused by two physical properties only: size of the emitter and the wavelength of the signal. The latter is influenced by the speed of sound in the medium and the frequency of the emitted signal.

Firstly, the **speed of sound** plays an important role. In air, due to the comparably low speed of sound, the constructive and destructive interferences are causing a higher directionality than in water where the sound speed is approximately four times larger (figures 1 and 2).

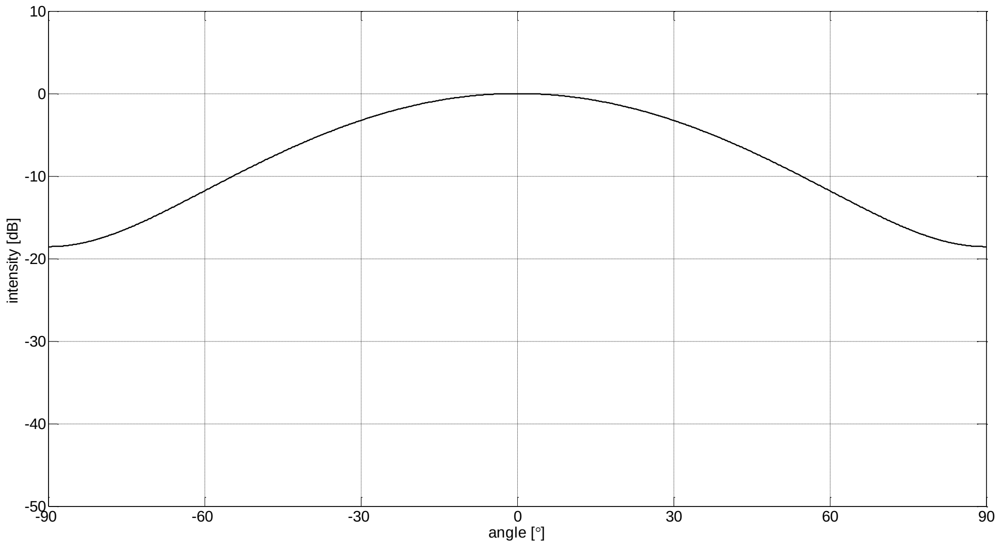


Figure 1: Sound field of a circular piston. Source parameters: size of emitter: 3.3 mm, frequency: 55 kHz, speed of sound: 340 m/s. This and the following figures have been plotted using matlab code provided by Peter Stilz.

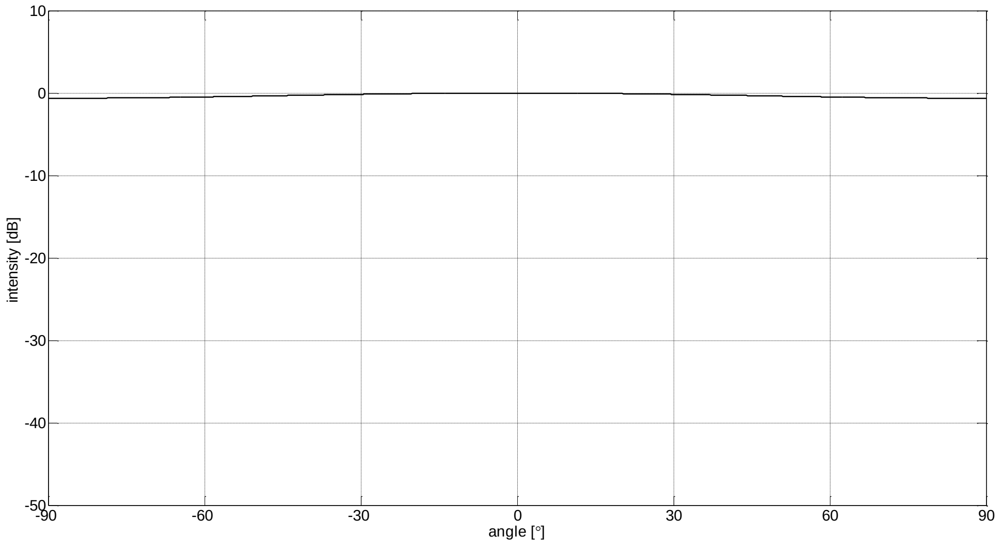


Figure 2: Same sized source as in Figure 1 emitting a signal with the same frequency, this time in water.

Secondly, the **frequency of the emitted signal** determines the directionality. The higher the frequency, the more directional the emission pattern (figures 3 and 4).

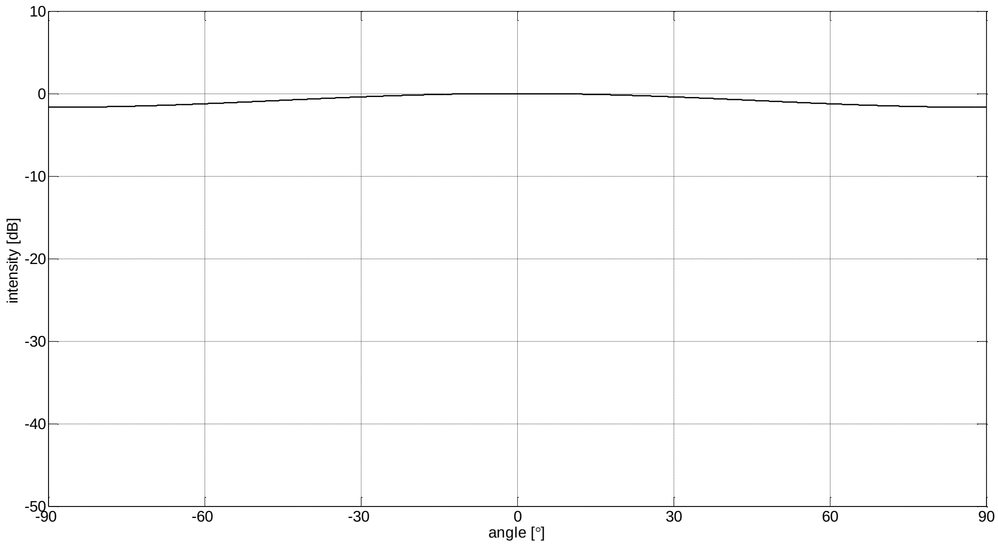


Figure 3: A simulated *N.noctula* emitting at 20 kHz in air (speed of sound: 340 m/s) with an assumed circular source with a radius of 3.3 mm.

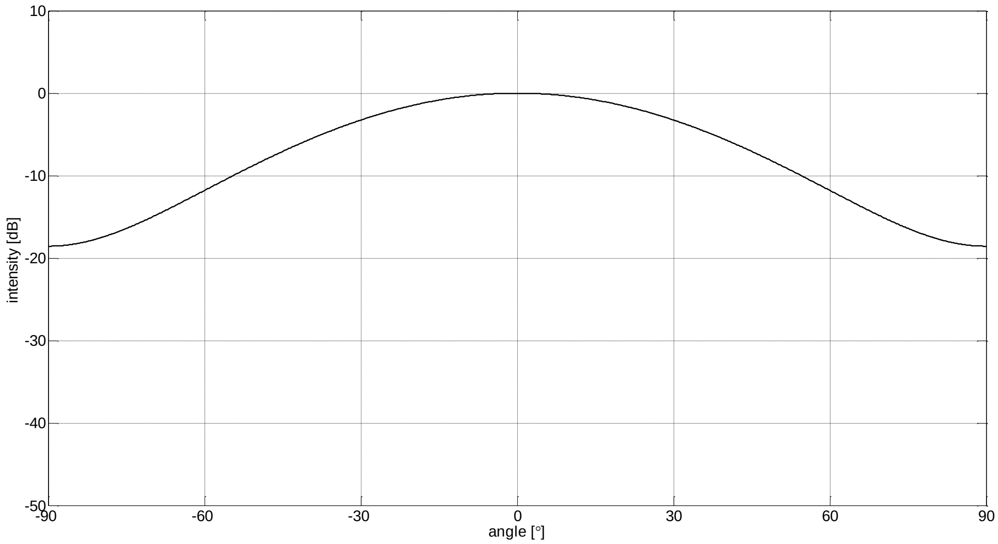


Figure 4: As frequency increases, so does directionality. Source parameters: radius of emitter: 3.3 mm, frequency: 55 kHz, speed of sound: 340 m/s. This would be a typical *M. daubentonii* beam pattern.

Thirdly, the **size of the emitter** influences directionality: The larger the transducer, the more directional the echolocation beam (figures 5 and 6).

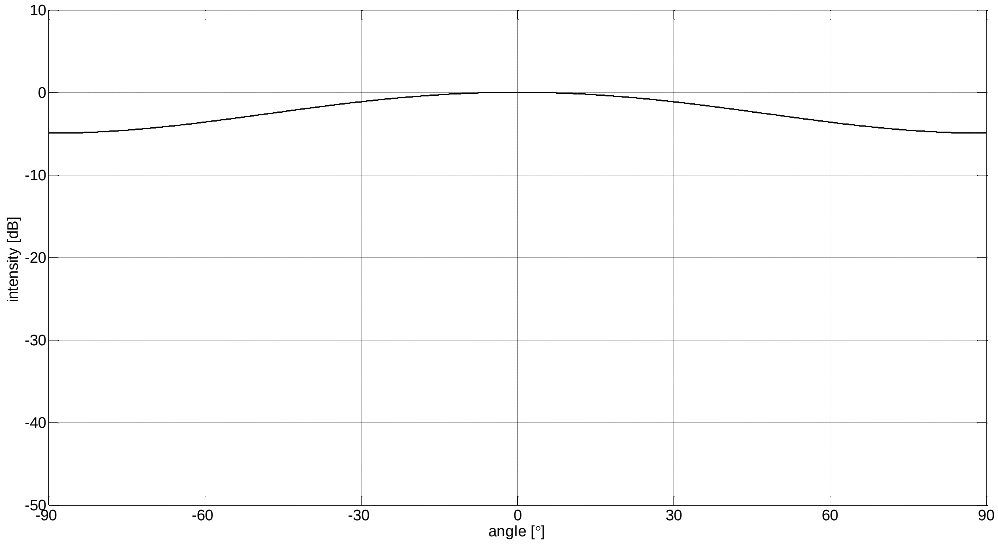


Figure 5: Simulated beam pattern at 55 kHz emitted by a circular source with a radius of 2 mm in air.

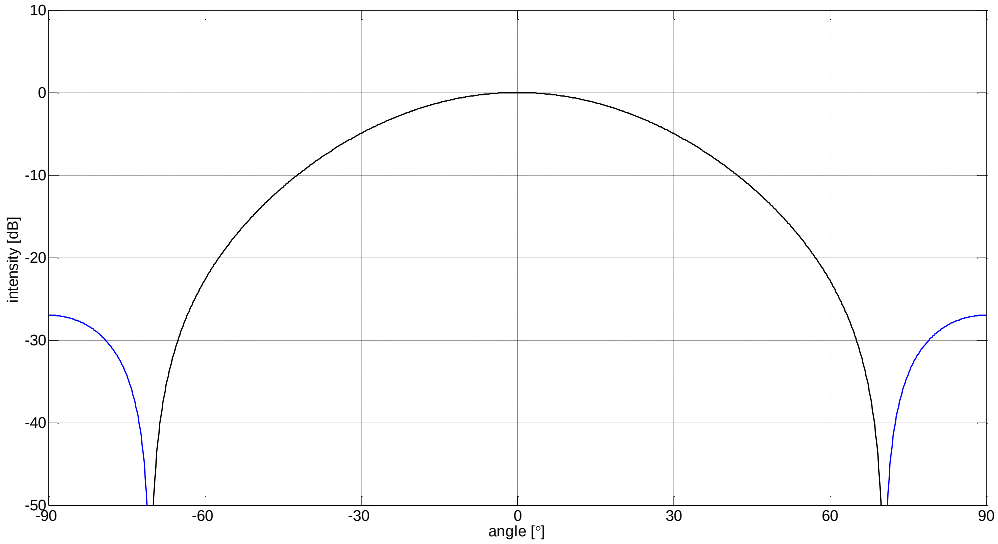


Figure 6: Simulated beam pattern by a circular source twice as large as the one in figure 5. Source parameters: radius of emitter: 4 mm, frequency: 55 kHz, speed of sound: 340 m/s. At this emitter size and frequency, first notches become apparent in the beam pattern at about 70° off axis.

4.1.3 Signal parameters in echolocating bats and toothed whales

4.1.3.1 Temporal pattern of signal emission in bats

Shortly after the discovery of echolocation as a sensory modality in bats, the temporal patterning of the echolocation signals was investigated. Individual bats adjust the pause between two echolocation signals according to the behavioral state they are in (Galambos and Griffin, 1942). Usually, one call is emitted per wing beat cycle, but as a bat approaches obstacles, background or potential prey items, calls are emitted in groups (dyads, triads, etc.). Simultaneously, the call duration is reduced. Griffin et al. (1960) defined three behavioral stages, search, approach and terminal phase based on the temporal pattern of call emission. This three stage model is still used today to describe the hunting and approach behavior of bats. Starting from the search stage, the first call emitted in reaction to a newly detected target marks the beginning of the approach stage (Schnitzler et al., 1987). It is indicated by a reduction of the pulse interval (PI). Finally, the terminal phase is divided into the so called Buzz I and Buzz II, both of which are comprised of a series of calls emitted at short PIs. During Buzz I, the PI undergoes a constant further reduction, whereas during Buzz II it remains constant at as low as 5 ms and in many species a decrease in the terminal frequency is observed (Kalko and Schnitzler, 1989).

Emitting more calls per time unit allows a better temporal resolution. However, bats are limited by the air, they need to produce calls at high intensity and at high repetition rate. A link between wingbeat and call emission has been shown by numerous studies (Schnitzler, 1970; Schnitzler, 1971; von Saalfeld, 1938). Other studies have linked call emission, wingbeat and respiration (Suthers et al., 1972). Calls are emitted during the upstroke, coinciding with expiration. Consequently, pauses between call emissions indicate inhalation. Considerable discussion is ongoing regarding the importance of the resulting call groups, also termed strobe groups. Some studies indicate a sensory advantage (Moss and Surlykke, 2001) whereas others link the grouping of calls to the pause needed for inhalation (Koblitz et al., 2010).

Calls are shortened as animals close in on targets or background. Due to the usually long echolocation calls and the comparably low speed of sound, shortening the calls when closing in on objects maximizes the overlap free window. The latter is the area in front of the bat where echoes are neither masked by the emitted call nor by returning background echoes (Schnitzler et al., 2003).

4.1.3.2 Temporal pattern of signal emission in toothed whales

Toothed whales do not inhale while diving. Air necessary to produce echolocation is stored in air sacs and clicks are produced by passing this air through the phonic lips from the two air sacs (ventral and dorsal). The amount of clicks that can be produced before recycling of air becomes necessary is much greater than in echolocating flying bats.

Similar to bats, emission rate varies as behavioral situation changes (figure 7). During search for prey, the inter click interval (ICI) remains constant and is reduced during the final buzz to as low as 2 ms in small odontoces (Miller, 2010; Wisniewska et al., 2012).

A similar patterning to strobe groups in bats has been observed in toothed whales in captivity (Finneran, 2013; Turl and Penner, 1989) and in free-ranging false killer whales and Risso's dolphins (Madsen et al., 2004a). The presumed advantages of this grouping are still debated.

In contrast to bats, signal duration in most toothed whales does not change as the animals approach an object, except for beaked whales which emit shorter clicks during the buzz (Johnson et al., 2006).

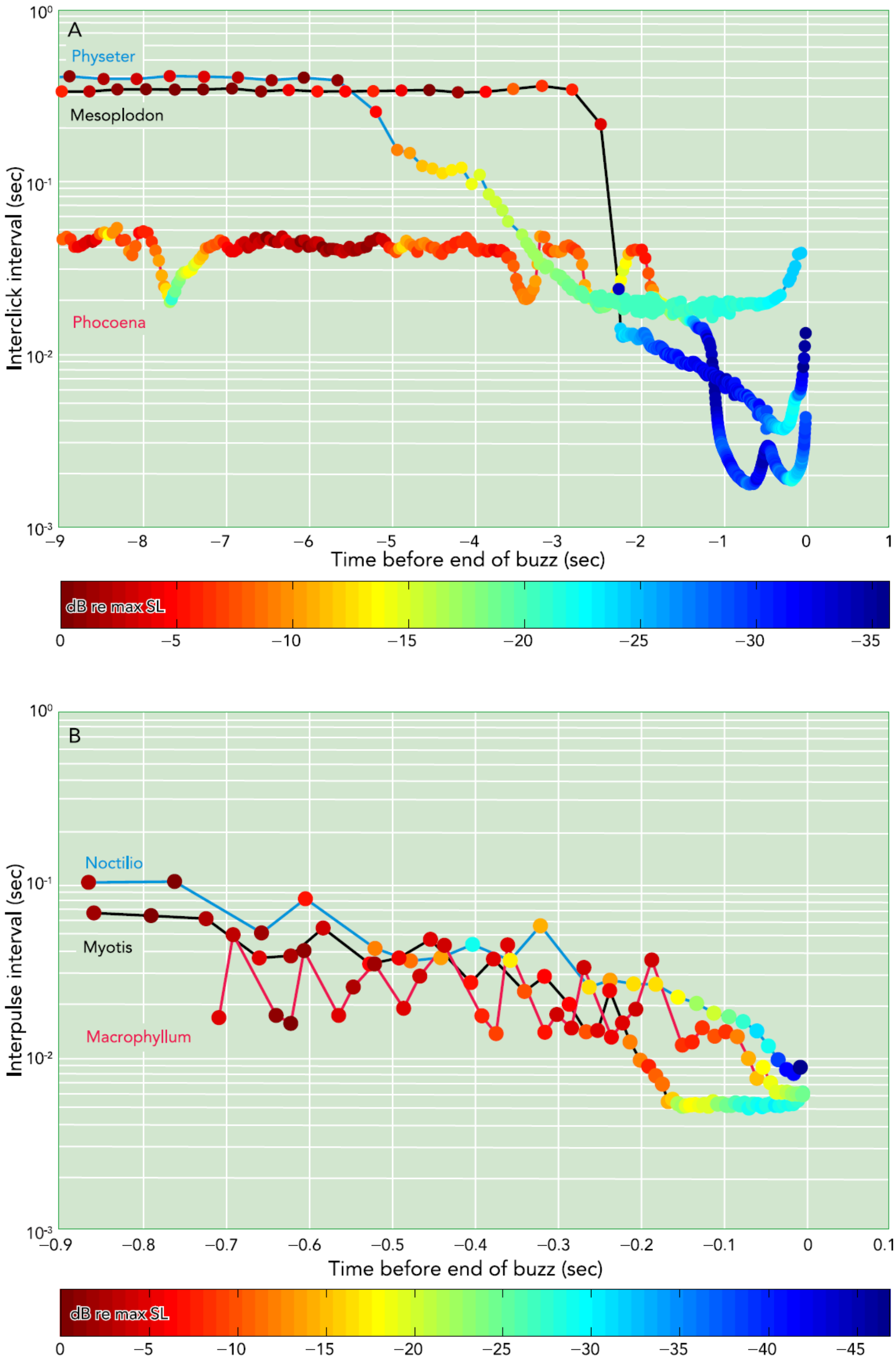


Figure 7: ICIs/PIs of toothed whales (above) and bats (below) [from Madsen and Surlykke (2013)].

4.1.3.3 Signal design in bats

Small bats in general emit higher frequencies (Jones, 1999). This has long been linked to their small gape size and hence their necessity to capture small insects which only reflect high frequencies. Only lately has the negative correlation between emitter size and call frequency been tied to constant directionality (Jakobsen et al., 2013d). High frequencies result in a high directionality; a large transducer has the same effect.

The taxa of microchiroptera are diverse and rich in species. It is divided into seven gilds based on a combination of the foraging habitat (open space, narrow space and edged space), foraging method (aerial, trawling and gleaning) and the prey detection mode (active echolocation, flutter detecting and passive listening) (Denzinger and Schnitzler, 2013; Schnitzler et al., 2003). The signal design can be used to assign any bat species to one of these seven guilds.

In addition, each species shows plasticity in echolocation call design depending on the behavioral stages, similar to the temporal patterning of call emission. When flying in open space, calls often lack higher frequency content and in some guilds have a long low frequency component. As bats approach an object of interest, they increase the bandwidth of their calls by additionally emitting higher frequencies. An exception to this is the call frequency of rhinolophids, hipposiderids bats and *Pteronotus parnellii*. These species emit long calls of mainly constant frequency (CF-calls). The emitted frequency is adjusted to the Doppler shifted echoes from stationary objects (Schnitzler and Denzinger, 2010) and no additional distance dependent frequency shifts has been observed.

4.1.3.4 Signal design in toothed whales

Odontocete clicks are considerably shorter than bat calls. Nevertheless, similar to bat species, odontocetes can be assigned to one of four groups based on their signal design (figure 8). The sperm whale uses low frequency clicks of high intensity and directionality (Møhl et al., 2003; Møhl et al., 2000) and forms its own group. The large group of whistling delphinids emits very broadband clicks, containing energy in frequencies even above 200 kHz (Rasmussen et al., 2015; Wahlberg et al., 2011c). Beaked whale clicks resemble bat calls the most as they are comparably long and frequency modulated (FM) signals (Johnson et al., 2006; Zimmer et al., 2005). The fourth and last group is a diverse group of at least 13 species: The six species of Phoconoidea (Kyhn et al., 2013; Villadsgaard et al., 2007), one (but possibly both) of the two species of Kogiiadae (Madsen et al., 2005) and six species of non-whistling delphinids of the Cephalorhynchus and Lagenorhynchus families (Kyhn et al., 2010; Kyhn et al., 2009). Calls emitted by members of this group are of narrow bandwidth around 130 kHz, resulting in the name narrowband high frequency (NBHF) clicks.

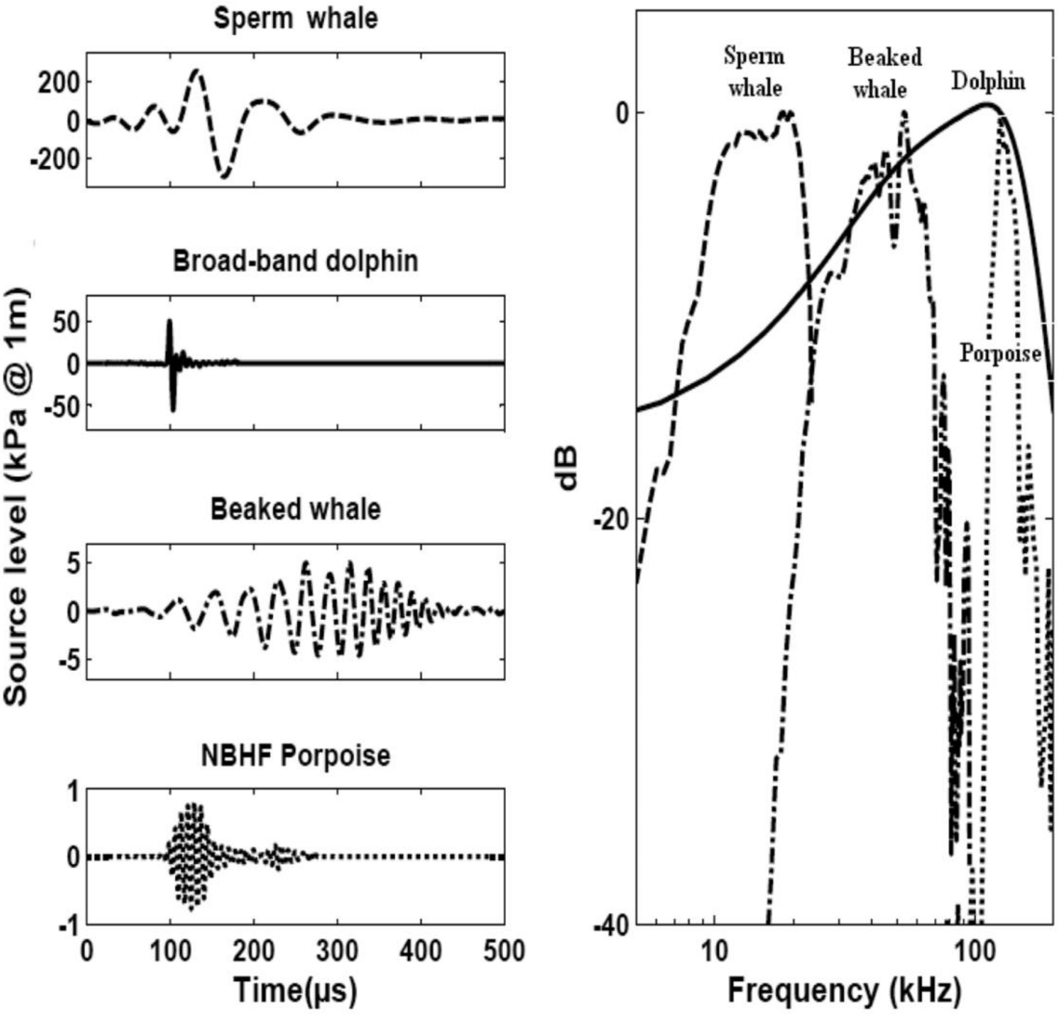


Figure 8: The four different groups of toothed whale biosonar signals [from Wahlberg et al. (2011a)]

4.1.3.5 Signal intensity

Both taxa emit signals of high intensity and in both taxa studies carried out with animals in captivity the source levels utilized by bats and toothed whales (Surlykke and Kalko, 2008; Villadsgaard et al., 2007). When the emission intensity is measured on the acoustic axis, it is referred to as the source level (SL). If the recording angle relative to the acoustic axis is unknown, the term apparent source level is used (Møhl et al., 2000) to describe signal intensity. Employing signals of high intensity is advantageous to the animal as these increase the detection distance. However, when foraging on eared prey that shows evasive reactions to echolocation signals, such as eared moths do, using echolocation signals of lower intensity (termed stealth echolocation) is advantageous as the prey item can be detected before reacting to the incoming call (Goerlitz et al., 2010). As animals of both taxa approach objects, the corresponding echoes increase in amplitude as distance decreases. The increase is usually expressed in dB increase per halving of emitter to reflector distance or by the formula X*log(distance). The magnitude of this increase depends on the extent of the reflector and ranges from 6 dB per halving of distance [20*log(distance)] of echoes from an extended target to 12 dB increase [40*log(distance)] of echoes from a small target. Constant emission intensity will consequently result in a steady increase of echo amplitude as animals approach prey items or obstacles. Interestingly both taxa have similar mechanism to prevent an overload of the auditory system. The emission intensity is reduced by approximately 6 dB per halving of distance leading to a constant received level at the reflector (Au and Benoit-Bird, 2003; Hartley et al., 1989; Kobler et al., 1985; Koblitz et al., 2011). In addition the sensitivity of the auditory system is reduced by an additional 6 dB per halving of distance (Hartley, 1992a; Hartley, 1992c; Linnenschmidt et al., 2012a). By this combination, the received echo is kept approximately constant during the approach.

4.1.3.6 Spatial coverage by echolocation in bats and toothed whales

Both bats and toothed whales emit their echolocation signals in a directional, forward oriented, so called sonar beam. The resulting beam width, parameterized as the -3 dB beam width (BW) defines the angle between the directions at which the intensity is halved compared to the on-axis intensity. In bats this has mainly been measured using stationary animals, one reference microphone and one microphone probing the sonar beam at different angles (Hartley and Suthers, 1987; Hartley and Suthers, 1989; Hartley and Suthers, 1990; Henze and O'Neill, 1991; Hiryu et al., 2006; Schnitzler and Grinnell, 1977; Shimozawa et al., 1974). The directionality of different odontocete species has mainly been measured of stationary animals, echolocating at a linear array of hydrophones (Au et al., 2012; Au et al., 1978; Au et al., 1999; Au et al., 1986; Au et al., 1995; Au et al., 1987; Branstetter et al., 2012). Directionality was higher for toothed whale clicks than for bat echolocation calls. The beam measurements of one harbor porpoise however showed that this species had the widest beam of all toothed whales (Au et al., 1999).

4.2 Array designs to study echolocating bats and toothed whales

4.2.1 Array designs to measure the directionality of stationary animals

4.2.1.1 Bats

The very first measurements on the directionality of bat calls were made using only a single receiver and well trained bats perching (Möhres, 1953; Schnitzler, 1968) or flying towards an offered prey item (Möhres and Neuweiler, 1966). A little later, a first microphone array was already used by Simmons (1969) and consisted of four microphones. *E. fuscus* and *C. rubiginosa* were trained to sit on a platform and echolocate in the direction of the microphone array. A decade later, a three microphone array was used to measure the beam width of a fixated *M. daubentonni* (Mogensen and Møhl, 1979). However most of the studies at that time used two microphones to measure the emission directionality of stationary animals. Bats were at first fixated by a screw or other holding device attached to the head to obtain recordings from a known position and head orientation. Beam measurements were then made with one reference receiver in front of the animal and one receiver moving around the animal (Hartley and Suthers, 1987; Hartley and Suthers, 1989; Hartley and Suthers, 1990; Henze and O'Neill, 1991; Hiryu et al., 2006; Schnitzler and Grinnell, 1977; Shimozawa et al., 1974). In some cases, electrodes were used to elicit echolocation calls (Henze and O'Neill, 1991; Shimozawa et al., 1974). Only recently much larger and two dimensional arrays have been developed to measure the beam width of stationary bats (Gaudette et al., 2014).

4.2.1.2 Toothed whales

The common experimental setup to study the directionality of toothed whales was to train an animal to go into a hoop, to a chin cup or bite plate positioned at a known distance to a hydrophone array and to echolocate towards it. First and mostly **linear arrays** were used to measure the directionality of stationary toothed whales either in the horizontal or vertical plane (Au et al., 2012; Au et al., 1978; Au et al., 1999; Au et al., 1986; Au et al., 1995; Au et al., 1987; Branstetter et al., 2012; Evans, 1973). However, linear arrays only provide information about the beam width and scanning direction in one dimension, namely the direction of array. Moreover, measuring the directionality of a beam that is not pointed directly at the array will result in an incorrect estimation of the directionality or beam width. Moore et al. (2008) were the first to measure the vertical and horizontal beam width simultaneously using a **two dimensional hydrophone array** with 24 receivers. Later a similar but larger system (29 receivers) was used to investigate the frequency-dependent variation of a dolphin’s beam pattern (Starkhammar et al., 2011). Since then, two dimensional arrays have been used more frequently to study horizontal and vertical directionality of stationary animals simultaneously (Ibsen et al., 2012; Kloepper et al., 2012a; Kloepper et al., 2012c; Koblitz et al., 2012) or to investigate SL regulation (Linnenschmidt et al., 2012c).

4.2.2 Array designs to measure sonar parameters of free ranging bats

A sophisticated **flight room array** was developed by Kaushik Ghose during his PhD thesis (Ghose, 2006). Starting with a linear 16 microphone array covering three walls of a large flight room, the beam width, prey competition and especially the scanning behavior of bats was studied in great detail (Chiu et al., 2010; Falk et al., 2011; Ghose et al., 2006; Ghose and Moss, 2003; Ghose and Moss, 2006; Ghose et al., 2009; Surlykke et al., 2009d). The position of the flying bats and prey items was determined with stereo infrared (IR) video recordings. Adding a vertical array of four additional microphones on one of the three walls allowed the measurement of the vertical beam pattern (Ghose et al., 2007). Later the vertical array was removed and a four microphone horizontal array covered the remaining wall, resulting in all four walls covered with a large horizontal array of 20 microphones (Yovel et al., 2010; Yovel et al., 2011).

A different setup was developed by Wiebke Pflästerer during her doctorate at the University of Tübingen, consisting of a **planar 16 microphone array** – basically a wall of 16 microphones arranged in a regularly spaced grid. This was used to study the scanning movements of Natterer's bats in the lab (Pflästerer, 2007). I used the same setup to study the SL reduction of big brown bats during landing approach (Koblitz et al., 2011; Koblitz et al., 2010).

The largest and most **complex flight room array** to date has been established recently by Matsuta et al. (2013). A total of 31 microphones cover three walls and parts of the floor and ceiling of a flight room. The bat's position is determined by stereo high-speed IR video recordings and the bat is equipped with a Telemike (a small microphone mounted to the bat's back) recording the echolocation calls close to the source.

4.2.3 Array designs for acoustic localization

Arrays have been used since 1963 to localize and track baleen whale vocalizations, first in two dimensions (Walker, 1963), later in three dimensions (Watkins and Schevill, 1972). Their low frequency calls propagate far and are emitted almost omni-directional. In addition due to the low frequency, these signals require only a low sampling rate when recording them, making them good candidates for long distance array recordings. However for precise source localization, high frequency signals are better suited. Localizing echolocators utilizing higher frequencies possesses two main challenges: The high frequencies are strongly reduced in amplitude by absorption, especially in air. In addition, high frequencies are usually emitted more directional than low frequencies making it less likely to record the same echolocation signal on multiple receivers.

For a long time, determining the position of bats in the field was mostly accomplished by stereo-photogrammetry or -videogrammetry (Kalko and Schnitzler, 1989; Kalko and Schnitzler, 1993; Schnitzler et al., 1987; Surlykke and Kalko, 2008). Acoustic localization of toothed whales was historically of greater importance than acoustic localization of bats due to the fact that there were no alternatives to determine the position of freely moving toothed whales. By measuring the time of arrival differences (TOAD) between the different receivers, the source position of a sound can be computed. A linear setup of receivers can only be used to determine distance to the array and the position in the dimension of array aperture. A vertical linear array can hence be used to determine distance to and depth of a source, a horizontal linear array to determine distance and horizontal displacement. In the remaining dimension, rotational symmetry around the array axis has to be assumed.

4.2.4 Array designs to localize an echolocator and to measure sonar parameters

The potential of using multi-receiver arrays to both localize an animal and analyze the acoustic parameters of its signals makes such setups an extremely powerful tool for bioacoustics research. Using three receivers in a linear configuration allows the computation of the distance between the animal and the array. Such a linear minimum receiver number array (MINNA) results in one analytical position for the source and thus an error estimate or validation is not possible. Such a system can be used to calculate the distance dependent transmission loss (TL) and based on this and the maximal received level (RL) of the three channels the SL of the emitted signal can be obtained. Adding at least one more receiver results in an over determined (ODA) linear array which additionally allows assessing the localization error. Only later arrays were also used to measure additional signal parameters such as directionality.

4.2.4.1 Bats

The first array used to study bats in the field was a large **horizontal linear array** with three receivers. Two of these receivers were microphones, the third was a biological microphone: the tympanic organ of a noctuid moth (Roeder, 1966). Almost 30 years later Surlykke et al. (1993) used a small linear array of three receivers with an aperture of 0.5 m. Based on the TOADs the distance to the bats could be determined and the ASL could be calculated. A similar but three times larger array with three receivers was used in a comprehensive study on the SL and its variation of 11 bat species (Surlykke and Kalko, 2008). Advancing from the three microphone horizontal array, one additional receiver was added above the middle microphone resulting in a **reverse T array** (Surlykke et al., 2009a), allowing for a 3D localization of the animal with an ambiguity at the plane of the array. This ambiguity is not crucial when making recordings in air since microphones are no omni-directional receivers. They record best on the acoustic axis, i.e. in front of the membrane. Sound impinging from behind the microphone is recorded by at least 20 dB less. This receiver directionality in combination with the high emission directionality of bats allows discriminating quite easily if a signal was emitted in front of or behind the array. This **reverse T array** was used to study directionality in the field (Surlykke et al., 2009a) and SL in the lab (Brinkløv et al., 2009) and field (Brinkløv et al., 2010). Soon, this array was even expanded further to a **plus array** with up to 12 microphones and the directionality of numerous bat species was studied in flight rooms (Brinkløv et al., 2011; Jakobsen et al., 2013a; Jakobsen et al., 2012; Jakobsen et al., 2013d; Jakobsen and Surlykke, 2010; Surlykke et al., 2013).

Far ahead of his time was Roland Aubauer when he designed an array system by combining two four receiver arrays, positioned at several meters distance to each other (Aubauer, 1995). In both arrays, four microphones were arranged in a **symmetrical star** arrangement with one central receiver and three receivers extending away from the center by 61 cm, with an angular spacing of 120°. Each of the two arrays resulted in a vector pointing towards the source which was consequently located where both vectors crossed. By placing the two arrays several meters apart, the array aperture is increased but the accuracy of the receiver position is maintained. This system was used in many studies (Goerlitz et al., 2010; Grodzinski et al., 2009; Holderied et al., 2006; Holderied and von Helversen, 2003; Schul et al., 2000). Later, this array concept was further advanced by using four **symmetrical star** arrays, one set up at each corner of the area where bats were recorded and localized (Fujioka et al., 2011).

The only **vertical linear array** ever used to study bats was developed by Jensen and Miller to investigate the influence of flight altitude on echolocation signals in the field (Jensen and Miller, 1999).

During the course of my doctorate the author converted the **planar 16 microphone array** into a field array which was used to investigate the scanning behavior and direction of call emission by localizing bats and measuring sonar parameters (Seibert et al., 2013; Seibert et al., submitted)

4.2.4.2 Toothed whales

Two array types have been used extensively to first localize free-ranging toothed whales and then perform signal parameter measurements: A **linear array** with three or more receivers and a four receiver **star array**.

Using three receivers in a **vertical linear array** allows the computation of the distance to the array and depth and has been used to measure the SL of free ranging narwhals, pygmy killer whales, false killer whales and Risso's dolphins (Madsen et al., 2004a; Madsen et al., 2004d; Møhl et al., 1990). Adding one more receiver results in an over-determined array allowing to compute one more TOAD than needed to determine the distance and depth and hence enabling to validate these two source coordinates. This array configuration has been used to measure the SLs of harbour porpoises (Villadsgaard et al., 2007), killer whales (Holt et al., 2009), bottlenose dolphins (Jensen et al., 2009), hourglass and Hector's dolphins (Kyhn et al., 2009). The same array setup has been used to measure the directionality in addition to the SL of bottlenose dolphins (Wahlberg et al., 2011c), Irrawaddy river, and Ganges river dolphins (Jensen et al., 2013). A larger six element vertical array has been used to perform the same measurements on Peale's and Commerson's dolphins (Kyhn et al., 2010), and harbor and Dall's porpoises (Kyhn et al., 2013).

A different configuration of four hydrophones, the **star array** (based on Aubauers design), was used to study SLs and changes thereof in relation to the animal-array distance of white-beaked dolphins, Atlantic spotted dolphins, killer whales and dusky dolphins in the field (Au, 2004; Au and Benoit-Bird, 2003; Au et al., 2004; Au and Herzing, 2003; Au and Würsig, 2004; Rasmussen et al., 2002; Schotten, 1998; Schotten et al., 2004; Simon et al., 2007) and of three harbor porpoises in captivity (Atem et al., 2009). The star array has once been used to measure the directionality of one species of dolphins, namely the white-beaked dolphins (Rasmussen et al., 2004).

Recently, larger **two dimensional arrays** have been used to study the directionality and changes thereof in free swimming harbour porpoises in captivity (Koblitz et al., in prep-d; Wisniewska et al., in prep).

A **large linear ODA** with ten receivers and fiber-optic cable was deployed at a depth of up to 950 meters to study the directionality of sperm whale clicks (Heerfordt et al., 2007). A smaller linear array consisting of five receivers was used to measure the directionality of northern bottlenose whales (Wahlberg et al., 2011a).

A **large non-linked array** was used extensively to study the comparatively low frequency but high intensity and high directionality clicks of sperm whales (Møhl et al., 2001; Møhl et al., 2003; Møhl et al., 2000; Wahlberg, 2002; Wahlberg et al., 2001). Three to seven different platforms (yachts and zodiacs) where used, each equipped with a Global Positioning System (GPS). One to three of the ten receivers were deployed from each of the platforms and their GPS was used to synchronize recordings and determine the positions of the platforms during post processing.

4.3 Considerations for array designs

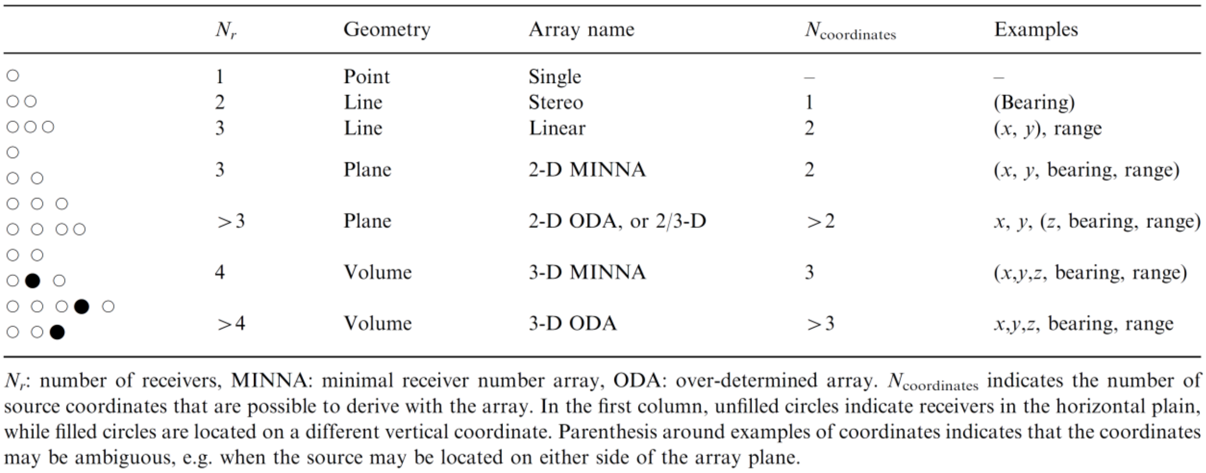
4.3.1 Array design and array size

The array design of choice depends on the parameters to be investigated and whether a stationary or moving animal is to be studied. Studying stationary animals, acoustic localization is not required and a narrowly spaced 2D array is the optimal solution when interested in e.g. detailed beam measurements (Gaudette et al., 2014). Using a linear array only is not an optimal setup, unless it can be assured that the beam center is directed at the linear array.

One of the arrays used during this doctorate, was a plus shaped design. It was used to measure the beam width of a stationary animal (Koblitz et al., 2012). Such a setup yields a good angular resolution in all four directions. However, this only applies if the beam is directed at the center receiver of the array. Stationary animals that still scan with their beam result in a lot of off-axis recordings that have to be discarded when working with this array setup.

In most cases, the echolocator has to be localized in order to perform the required measurements. In this case, the maximal dimension (aperture) of the array matters, as this restricts the maximal distance at which the animal can still be localized. As a rule of thumb, an animal can be localized at distances smaller than tenfold the array aperture. Hence, when studying animals far away, the receivers need to be spaced wide apart.

When using acoustic localization, the number of receivers arranged in a linear, 2D or 3D arrangement influences which source coordinates can be measured (table 1).



	N_r	Geometry	Array name	$N_{\text{coordinates}}$	Examples
○	1	Point	Single	–	–
○○	2	Line	Stereo	1	(Bearing)
○○○	3	Line	Linear	2	(x, y), range
○ ○ ○	3	Plane	2-D MINNA	2	$(x, y$, bearing, range)
○ ○ ○ ○ ○ ○○	>3	Plane	2-D ODA, or 2/3-D	>2	x, y, (z, bearing, range)
○ ○ ○● ○	4	Volume	3-D MINNA	3	$(x,y,z$, bearing, range)
○ ○ ○● ○ ○ ○●	>4	Volume	3-D ODA	>3	x,y,z, bearing, range

N_r: number of receivers, MINNA: minimal receiver number array, ODA: over-determined array. $N_{\text{coordinates}}$ indicates the number of source coordinates that are possible to derive with the array. In the first column, unfilled circles indicate receivers in the horizontal plain, while filled circles are located on a different vertical coordinate. Parenthesis around examples of coordinates indicates that the coordinates may be ambiguous, e.g. when the source may be located on either side of the array plane.

Table 1: Receiver configuration and source parameters that can be measured (Madsen and Wahlberg, 2007).

In most studies investigating the SLs of odontocetes, **vertical linear arrays** have been used (Jensen et al., 2009; Kyhn et al., 2010; Kyhn et al., 2009; Villadsgaard et al., 2007). A three to four element array is sufficient to measure distance and depth of the echolocating animal, thus allowing accounting for transmission loss. However, using this setup can lead to a typical error when estimating the beam width: Horizontal scanning of the animal over a vertical array will result in varying measured beam widths depending on whether the center of the beam is directed at the array or if the array is only tangent by the beam. Some post processing methods have been used to reduce the influence of this error on beam and SL measurements (Madsen and Wahlberg, 2007).

When all three coordinates of the animal need to be known, the simplest approach is the **reverse T-shaped array** or the **star array**. In one recent bat study we chose the reverse T setup (Götze et al., in prep), the same design successfully used by Surlykke et al. (2009a) and Surlykke and Kalko (2008). This array allows determining all three coordinates with an ambiguity at the array plane. By placing the array horizontally one meter above the ground we could eliminate this ambiguity. This array performed well in animal tracking. However, beam measurements and even SL measurements were impossible due to the orientation of the array relative to the bats flight movements.

A **large planar array** is a good array setup to combine acoustic localization with detailed beam measurements. The narrower the receiver spacing, the better the angular resolution and the higher the likelihood to detect side lobes or other beam details. In most studies carried out during my doctorate, such an array arrangement with 15 or 16 receivers was used (Koblitz et al., in prep-d; Seibert et al., 2013; Seibert et al., submitted).

4.3.2 Avoiding echoes

By using Polyethylene (PE) for under water arrays, echoes from the array can be reduced or even avoided since this plastic has almost the same impedance as water and can thus be regarded as acoustically transparent. Placing the receivers in front of the main array structure additionally reduces the effect any potential echoes might have by increasing the signal-echo delay. When recording bats, pieces of anechoic foam can be placed between the array structure and the receivers to reduce echoes from the array frame.

4.3.3 Recording equipment

4.3.3.1 Receivers

When acoustic localization is the main purpose of the array, receivers with a large dynamic range are the key factor to increase the detection distance and, by obtaining recordings with a good signal to noise ratio (SNR), the quality of the cross correlation. A good receiver for the acoustic localization of bats is the Knowles SPM0204UD5 or the PC-Tape microphone designed by the department of animal physiology at the University of Tübingen.

The most cost-efficient receivers for the acoustic localization of odontocetes are self-made (Wisniewska et al., 2012). A more expensive but very reliable alternative are the calibrated Reson hydrophones.

More care needs to be taken when the same receivers are additionally used to measure the RL very precisely in order to determine the beam width. The size of the receiver (including housing or holding) relative to the wavelength is of great importance to avoid reflections or refractions of sound. In addition, the directionality of the receiver is important as it needs to be considered during data processing. The most perfect receiver for beam measurements would be truly omni-directional. Since we used a directional receiver, the Knowles FG-23329, we determined the frequency dependent directionality of every receiver during calibration. When analyzing the recordings made in the field, the angular position of the bat relative to any of the receivers in the array and hence the directional frequency response was compensated for. For receivers which are rotationally symmetric, such as the Knowles FG-23329, this is a feasible approach. With non-rotationally symmetric receivers this process is cumbersome.

When the array is set up and dismantled on a regular basis, fixing the receivers' positions has been proven very useful and time saving. In the large array used to record bats, the microphones were attached to 1 mm diameter nylon fishing line (figure 9, 10).

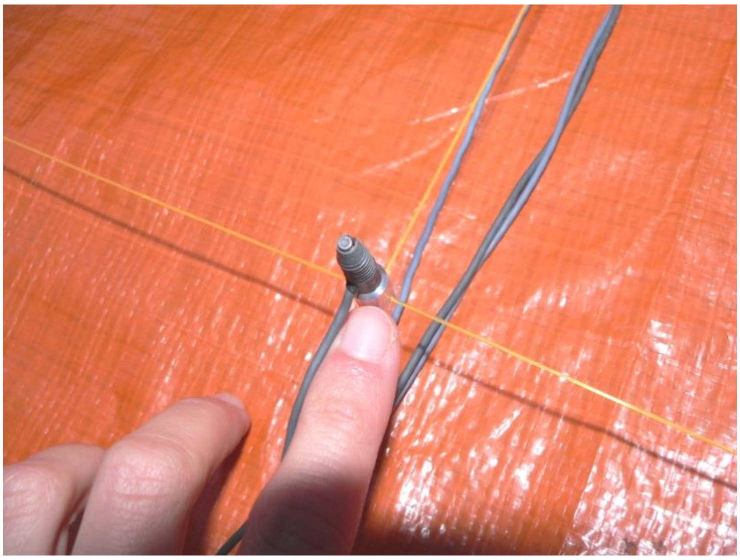


Figure 9: One array microphone (small silver circle), holding device (grey plastic and aluminum casing), cable (grey) and nylon fishing line. Photo: Anna-Maria Seibert.

Figure 10: Array setup: 4x4 m aluminum frame with fishing line grid, holding 16 microphones where the fishing lines intersect. Photo: Jens Koblitz.

4.3.3.2 Amplifier

The cables between receiver and amplifier should be kept as short as possible to keep the SNR at a maximum and to avoid any cross talk. The amplification needs to be chosen according to the receiver sensitivity, the expected distance to source, the SL thereof and the input range of the analog to digital converter (ADC).

4.3.3.3 Analog-to-digital-converter

The higher the sampling rate, the more accurate can the position of a source be determined. The common sampling rates today are between 250 kHz and 1 MHz. For most parts of this doctorate, a sampling rate of 500 kHz and 16 bit was used.

Recording with multiple receivers at high sampling rates and a 16 bit resolution results in two new potential bottlenecks: Transferring the data from the ADC to the computer and then storing it.

The National Instruments PXI-Express Card 8360 used for most parts of this thesis has a data rate of up to 110 MB/s. One receiver, sampled at 500 kHz with 16 bit resolution results in approx. 1 MB/s, so theoretically data from up to 110 receivers could be transferred. The computer’s central processing unit CPU is usually not limiting, unless real time spectrograms need to be computed. The data writing speed of the rotating hard disc drives (HDDs) was a limitation until recently. HDDs can write data between 50-150 MB/s, but the speed decreases as the HDD fills up. The recent development of solid state discs (SSDs) will overcome this bottleneck; these non-rotating media can store data at more than 200 MB/s and the speed is independent of remaining free storage.

4.4 Using acoustic localization

For the study presented in chapter 5.1, the position of the bats was determined using stereo videogrammetry, where the moving animal was recorded with two cameras at fixed positions. After calibration of the overlapping field of view, the sound source (i.e. the head of the bat) was marked in both video recordings frame by frame. The animal's position and the position of other objects (i.e. the array receivers) were determined in the same way.

As explained in chapter 5.4, a porpoise was trained to stay at a known positon at a fixed distance from the array, making transmission loss and beam measurements relatively easy.

For all other chapters presented in this thesis, acoustic localization was used to determine the position of the echolocating animal. The terms time of arrival difference (TOAD) and time difference of arrival (TDOA) are both common in the literature and are used synonymously. In the course of this thesis, the acronym TOAD will be used instead of the initialism TDOA.

Experimental acoustic localization to determine the track of an echolocating animal was performed as a four step process:

1. Determining each TOAD of the signal at the different receivers
2. Determining the position of the echolocator by
    a. graphical solution
    b. analytical solution
3. If necessary, separating signals from multiple echolocators and excluding echoes
4. Generating tracks of animals based on the localized positions of call emissions

4.4.1 Determining the TOAD

In few cases, the TOAD of a signal at multiple receivers can be determined by using the time of arrival of the onset of the signal or of the peak either by naked eye or by manual peak detection. However, either method requires a good SNR and/or a clear onset of the signal. Therefore, only for very few echolocation signal types it would be possible to determine the TOAD using this simple approach. All echolocation signals are directional and all delphinid signals show deteriorating waveforms and different spectral composition at different angles from the acoustic axis (and hence receivers). For bats, only CF species would be a potential candidate for this labor intensive and error prone approach.

The cross correlation (Helstrom, 1975) of signals or a method based upon cross correlation is usually the method of choice and I have used it throughout this thesis. A threshold based click detector is used in matlab to detect every signal above a certain threshold for one of the central array channels. A time window is then computed around this signal and the signal within that time window is used for the subsequent cross correlation. The window size needs to be chosen in accordance to the size of the array and the speed of sound to ensure that this window contains the signal at each receiver position. In addition, differences in signal length, signal repetition rate and occurrence of echoes have to be considered. The window needs to contain the signal in all channels but should exclude any subsequent echoes.

The time window containing the signal at one receiver is used as a template and then cross correlated with each time window of the other receivers or a subset of receivers. The x-axis value (i.e. the time instant) of the maximum of the cross correlation function is used as the TOAD of this receiver combination.

In Götze et al. (in prep), one of the four receivers was used as a template and cross correlated with the remaining three, resulting in three independent TOADs. When the planar 16 microphone array was used to study bats, one receiver was used as a template and cross correlated with the remaining 15, resulting in 15 independent TOADs (Seibert et al., 2013; Seibert et al., submitted). When studying free ranging odontocetes (Koblitz et al., in prep-d), a mixed approach was chosen: Due to the high directionality of the signal and the scanning behavior of the animal, not all receivers received the signal at a good SNR. Hence, varying receiver combinations (named sets hereafter) were used and for each set, the signal at one receiver was cross correlated with the remaining receivers of that set. For most signals, all sets yielded comparable localizations. However, especially when the template recording had a bad SNR, the localization was erroneous (see e.g. receiver set 6:12 in figure 11).

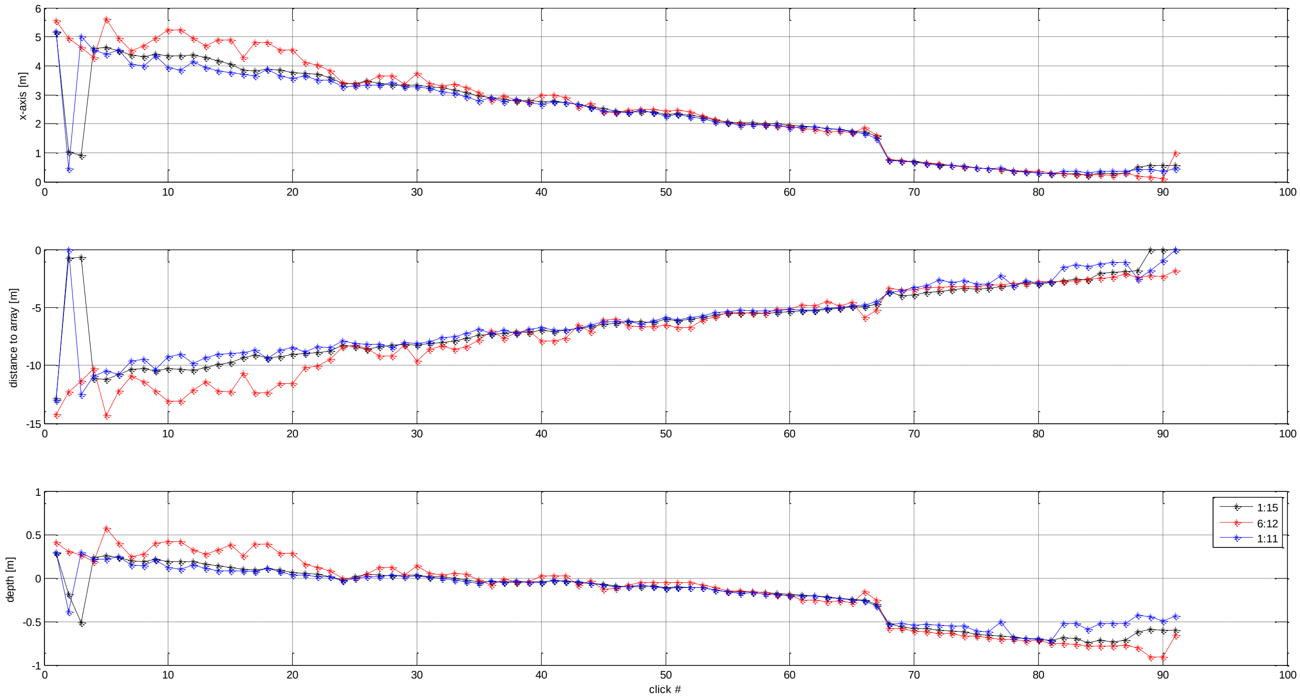


Figure 11: Source position for each of the 91 clicks (on x-axis) derived using three different sets of receivers, data from Koblitz et al. (in prep-d). Top panel: horizontal offset, middle panel: distance to array, lower panel: depth relative to topmost receiver.

4.4.2 Improving the quality of the TOADs

4.4.2.1 Filtering

A high pass filter is applied to all recordings in the first step of the analysis to reduce low frequency noise and a potential direct current (DC) offset. When using acoustic localization to position bats, it is crucial to consider the particular signal type emitted by that bat species. Cross correlating two pure tones of the same frequency results in a sinusoidal signal convoluted with a saw tooth signal. The cross correlation function is hence lacking a clear peak and CF bat calls need to be processed differently (see below). When analyzing calls of FM bats emitted in open space, these calls still contain a long part of constant frequency (the CF-tail). Localizing these animals is possible by applying narrowband bandpass filters to exclude the CF-tail from the cross correlation. Cross correlating only the FM part of such calls results in precise TOAD measurements.

4.4.2.2 Cross correlating the envelope

Different toothed whale species emit different echolocation clicks. The short broadband clicks of delphinid species are optimal to measure the TOAD. Animals in the NBHF group emit relatively long narrowband high frequency echolocation clicks. Cross correlating these clicks results in multiple good fits, i.e. a broad cross correlation function. Computing the envelope of the echolocation click and cross correlating this envelope results in a more stable and unambiguous TOAD (figure 12). Cross correlating echolocation clicks from other odontocete species, such as dolphins, belugas or narwhales does not require to cross correlate the envelope due to the click like nature of their echolocation signals.

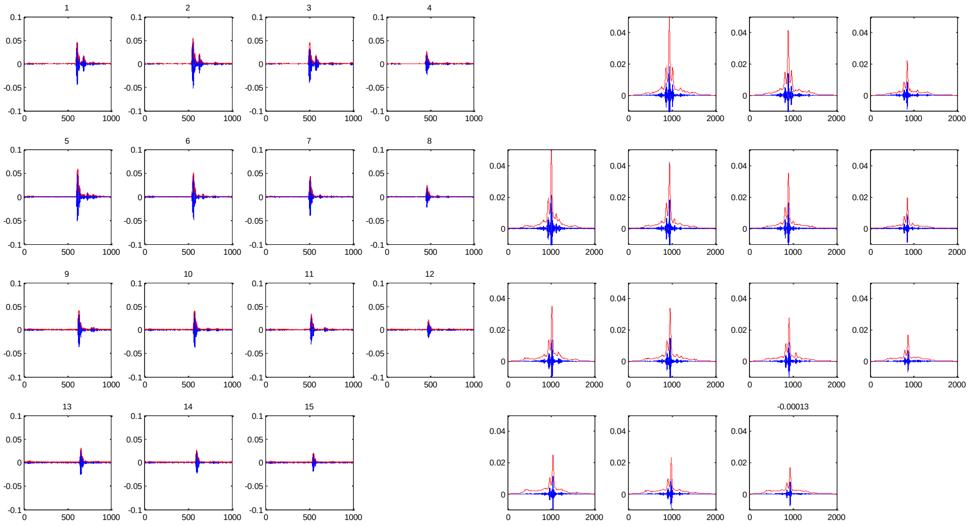


Figure 12: One porpoise click recorded at 15 receivers (blue lines) shown in the orientation in which they are arranged in the array. Receiver number one (upper left graph) is used as a template and cross correlated with the remaining 14 receivers. Each cross correlation function is plotted in blue on the right hand side of the figure. Cross correlating the signal results in a multimodal and broad cross correlation function. The broad cross correlation function is caused by the quasi sinusoidal signal, the multimodality is caused by an echo present in the template and in some of the other receivers. Cross correlating the envelopes (the red lines in the left graphs) results in a more pronounced peak in the cross correlation functions [see the red lines in the right graphs, Koblitz et al. (in prep-d)].

4.4.2.3 Resampling of the signal

Recordings made with a sampling rate of 500,000 Hz naturally result in a time resolution of 2 µs. By resampling the signal (i.e. interpolating between the discrete measurements made by the ADC, the time resolution in the following analysis is increased without adding additional information. This process is especially useful if recordings are made with a lower sampling rate, such as in Götze et al. (in prep).

4.4.2.4 Measuring the TOAD of CF-FM bats

Bats emitting (quasi) CF echolocation calls, e.g. greater horseshoe bats, are challenging to localize using experimental acoustic localization. These animals emit long calls with a duration of almost 100 ms and move at a considerable speed of at least 5 m/s (Tian and Schnitzler, 1997). Hence, the animals travel approximately half a meter while emitting a single call. In addition, the long constant frequency part of the call poses even greater challenges than NBHF species echolocation clicks. In order to localize these species, I first applied a narrowband filter and extracted only the FM part(s) of the signal. I then determined the position at the onset of the call (if the FM up-sweep was present in that particular call) and at the offset of the call (the FM down-sweep was always present in the obtained recordings) and computed the flight path accordingly.

4.4.3 Determining the position of the echolocator

In the second step of experimental acoustic localization, the TOADs are used to determine the position of the echolocator relative to the receivers either graphically and/or analytically.

4.4.3.1 Graphical solution

The TOADs between receivers can be visualized using hyperboloids. A hyperboloid describes a three dimensional surface on which the difference in distance between any point on the hyperboloid and two focal points is constant. Given two receivers with a known distance between them (the two focal points), the TOAD can be plotted as a single hyperboloid. Using three receivers results in three TOADs, two of which are independent (e.g. receiver pairs 1-2 and 1-3). These two hyperboloids can intersect; the intersection indicates the possible locations of the source. By adding more receivers, more hyperboloids are added, ideally reducing the usually curved intersecting line of the first two hyperboloids to a single point. Plotting these hyperboloids, and even more complex, the intersections of them was not attempted graphically within this doctorate thesis. For graphical representation the hyperboloids are usually sliced in the plane in which the source is analytically placed and the resulting hyperbolae are plotted. This reduces a 3D configuration to a 2D representation.

4.4.3.2 Analytical solution

For the analytical solution, firstly a matrix with receiver coordinates and secondly the TOADs are used to determine the sound source by Pythagorean geometry. The final position is determined using the least squares solution (Wahlberg et al., 2001). However, this analysis can result in unrealistic positions. It is therefore crucial to confirm the analytical solution by plotting the hyperbolae over the analytical solution (figure 13).

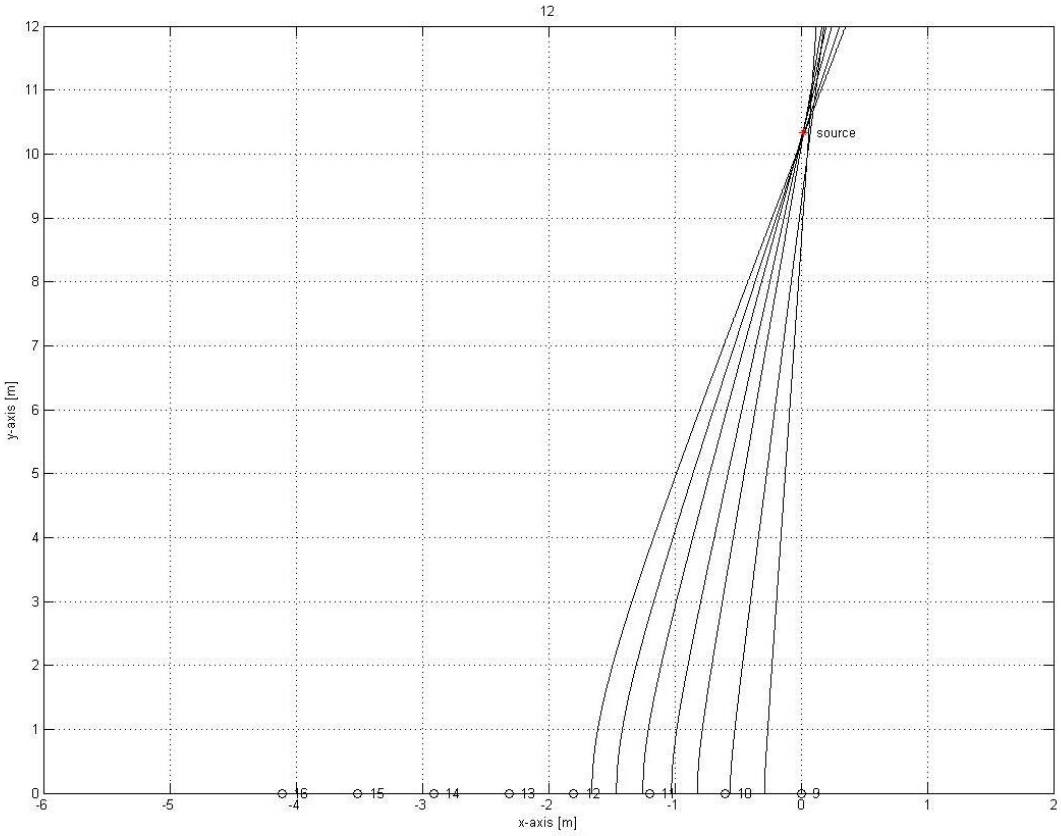


Figure 13: A horizontal array of eight receivers (marked by the circles on the x-axis) used to localize a porpoise [data from Wisniewska et al. (in prep)]. Receiver number 9 functions as the template and is cross correlated with the remaining 7 receivers, resulting in 7 TOADs and consequently in 7 hyperbolae. These hyperbolae do not intersect in one single point, however, all intersections are confined to a one by one meter space. The analytical source position (red star) was derived by using the least square solution of the equation system based on Pythagorean geometry.

4.4.4 Generating tracks of animals

When using more than one receiver set, the different sets have to be merged, excluding outliers due to e.g. bad SNR (figure 13). In addition, possibly localized echoes can be excluded by visual verification. All verified positions are then used as anchor points. The movement path of the animal is then interpolated and smoothed based on these anchor points. In order to still include signals in the subsequent analysis which originally had a SNR too low to be usable for localization, the software click detector is used again, this time at a lower threshold to include lower amplitude signals. The positions of the animal at emissions of such clicks is determined by again interpolating between the anchor points.

4.4.5 Separating signals from multiple echolocators

A major advantage of acoustic localization is the possibility to assign single signals to one of multiple individuals of the same species present (figure 14). This cannot be performed with any other method of localization.

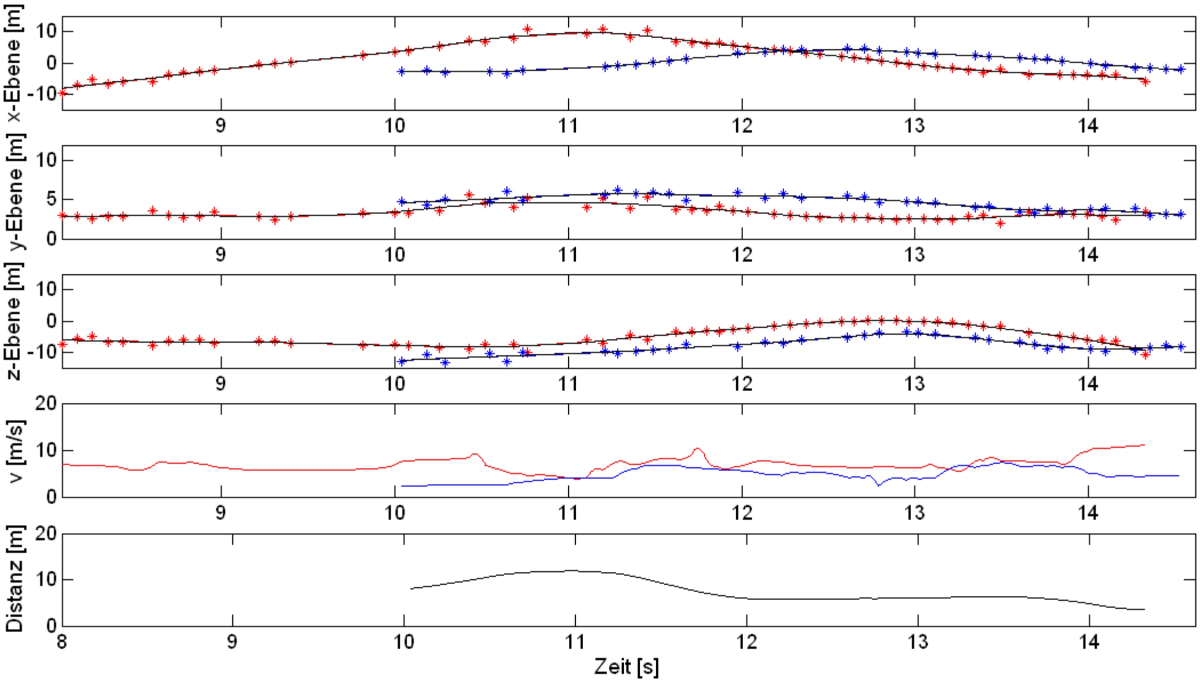


Figure 14: Acoustic localization results of a 6 second long recording plotted in the three dimensions [x,y,z, top three panels, data from Götze et al. (in prep)]. Echolocation signals are assigned to individuals by visual verification under the assumption that two animals cannot be in the same position at the same time and assuming reasonable flight speeds. Clear separation between animal one (red) and two (blue) was possible, especially based on the x and z dimension. The 3D flight paths of the two individuals (black lines in top three panels) can then be interpolates based on the assigned anchor points. The flight speed of each individual and the distance between the two can now be computed (lower two panels).

5 Manuscripts and publications

For the following papers by Koblitz et al., the experimental designs and methodologies as well as the results were discussed, and the manuscripts written together with Hans-Ulrich Schnitzler. Peter Stilz provided and adapted the matlab program SONARBEAM and contributed additional matlab code, was deeply involved in the discussion of the setup, the analyses, and results.

5.1 Source levels of echolocation signals vary in correlation with wingbeat cycle in landing big brown bats (*Eptesicus fuscus*)

This paper was published in the Journal of Experimental Biology in 2010. The data was recorded as part of my diploma thesis while the analysis was carried out at the beginning of my doctorate. Elena Kudryavitskaya tracked the bat's wing movement in the IR-video recordings. I recorded the data, performed most of the analysis and co-authored the paper.

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Figure 15: A big brown bat just before lifting off. Photo: Jens Koblitz

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Source levels of echolocation signals vary in correlation with wingbeat cycle in landing big brown bats (*Eptesicus fuscus*)

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SUMMARY

Recordings of the echolocation signals of landing big brown bats with a two-dimensional 16-microphone array revealed that the source level reduction of 7 dB per halving of distance is superimposed by a variation of up to 12 dB within single call groups emitted during the approach. This variation correlates with the wingbeat cycle. The timing of call emission correlates with call group size. First pulses of groups containing many calls are emitted earlier than first calls in groups with fewer calls or single calls. This suggests that the emission of pulse groups follows a fixed motor pattern where the information gained from the preceding pulse group determines how many calls will be emitted in the next group. Single calls and call groups are centred at the middle of the upstroke. Expiration is indicated by call emission. The pause between groups is centred at the middle of the downstroke and indicates inspiration. The hypothesis that the source level variation could be caused by changes in the subglottic pressure due to the contraction of the major flight muscles is discussed.

Key words: source level, respiration, timing of call emission, microphone array, bat, echolocation.

INTRODUCTION

Landing *Eptesicus fuscus* exhibit the same typical approach pattern as other bats, which is characterized by a switch from a single echolocation pulse to groups with two, three or more pulses when the bats close in on the landing site (Melcon et al., 2007). Studying the source level (SL, the sound pressure level a sound source would produce on the acoustic axis at a distance of one meter without atmospheric attenuation) of the approach signals with a 16-microphone array, we found that the SL is on average reduced by approximately 7 dB per halving of distance (J.C.K., unpublished observations). However, we also observed that the source level within and between groups varied considerably, resulting in an oscillating pattern. From this observed pattern, we derived the hypothesis that the variation pattern is correlated with the wing-beat cycle. Here, we investigate how the variations of signal SL during the approach to a landing site correlate with wingbeat.

Flying bats perform three motor processes simultaneously: flight, respiration and production of echolocation signals. During flight, sound emission is often correlated with the wingbeat and respiratory cycles. In this paper, we determine how the timing of call emission correlates with wing position and varies with group size. We use the correlation between sound emission pattern and wingbeat as an indirect indicator for the correlation between respiratory cycle and wingbeat. We then discuss the variations in signal SL in relation to the presumed respiratory cycle.

Past studies have shown that respiration and wingbeat are linked in a one-to-one relationship (von Saalfeld, 1938; Suthers et al., 1972). Inhalation in *Phyllostomus hastatus* occurs during the downstroke, expiration during the upstroke (Suthers et al., 1972). Bats approaching a target or obstacle emit groups of two, three or more calls, separated by a longer 'in between group pulse interval' (Galambos and Griffin, 1942; Kalko and Schnitzler, 1989). Grinnell and Griffin (Grinnell and Griffin, 1958) first proposed that this grouping of calls corresponds to the respiratory cycle. In experiments where sound emission and respiratory cycle were monitored in parallel, it was found that call emission is linked to the respiratory cycle in resting bats (Möhres, 1953; Schnitzler, 1968) and also for bats in flight (Suthers et al., 1972). Flying bats emit calls mostly during the upstroke, coinciding with expiration (Schnitzler, 1971; Suthers et al., 1972). Consequently, pauses between call emissions indicate inhalation (Schnitzler and Henson, 1980; Wilson and Moss, 2003). This has been confirmed by Lancaster and colleagues (Lancaster et al., 1995) who recorded diaphragmatic myopotentials from flying bats and showed strong evidence for inspiration during the pauses between call emission.

The timing of call emission relative to wing position has been studied in various bat species in the field and in flight rooms. Some of these experiments validate that sound emission, and probably expiration, extends from the end of the downstroke through the upstroke to the beginning of the downstroke. This was confirmed for *Myotis lucifugus* emitting single calls, dyads (groups of two calls) and triplets (Schnitzler, 1971) and for single calls and dyads emitted by *P. hastatus* (Suthers et al., 1972). Single calls of *Myotis daubentoni* and *Pipistrellus kuhli* flying in the field were emitted when the wings were around the upper turning position (Schnitzler et al., 1987; Kalko and Schnitzler, 1989). *Pipistrellus pygmaeus* flying in a flight room emitted single calls either at the end of the upbeat or at the beginning of the downbeat. Dyads were emitted at the end of the upbeat and at the beginning of the downbeat (Wong and Waters, 2001). *Mystacina tuberculata* emitted calls either at the end of the downstroke or early in the upstroke (Parsons et al., 2010). However, other studies presented different results. For instance, Kalko (Kalko, 1994), studying free-flying pipistrelle bats, describes a wingbeat pattern where most of the signals during search flight were emitted during the downbeat. Some studies show that bats sometimes override the tight coupling of wingbeat and call emission and emit calls at any wing position (Suthers et al., 1972; Lancaster et al., 1995; Moss et al., 2006). These discrepancies might indicate that the correlation between sound emission, wingbeat cycle and respiration is not as strict as assumed so far.

MATERIALS AND METHODS

Training and holding of bats

Four adult big brown bats (*Eptesicus fuscus*, Beauvois 1796, 3 males, 1 female) were trained to start from the experimenter's hand, fly across the flight room and land on a landing grid 5.4 m away. When no training or experiments were carried out, bats were held in holding facilities in a reversed day–night cycle (with lights on at 17:00 h, lights off at 09:30 h) at a constant temperature of 24°C and humidity of 70%. Water was provided *ad libitum*, supplementary vitamins were given in the form of Nutrival paste once per month. When bats were trained or when recordings were made, food (*Tenebrio* sp. larvae) was only given as a reward during sessions. During trials and training, the light in the room was turned off to exclude visual orientation.

Experimental setup and recordings

The walls and floor of the experimental room [6.0 m × 3.6 m × 2.9 m (length × width × height)] were covered with foam sheets to reduce echoes. The landing grid was positioned at a height of 1.3 m at the far end of the room. A planar microphone array, consisting of 16 Knowles FG-3329 electret microphones, arranged in a four-by-four grid was positioned vertically, forming one plane with the landing grid. The spacing between the microphones was 0.35 m, allowing the bat to land on the grid without touching the microphones closest to the landing grid. Absolute calibration of each microphone was achieved by comparison with a calibrated Bruel&Kjær 4138 microphone.

The flights of the bats were recorded with three Sanyo IRP infra-red-sensitive video cameras. Two cameras overlooked the entire flight of the bats, the third camera was used to determine the exact time of landing. Video recordings were made with 25 (interlaced) frames per second, and each half-frame was illuminated with a flash of duration 1 ms from an infra-red strobe system at a rate of 50 Hz, resulting in a pin-sharp picture of the bat in flight. Recordings were digitalized using Sony DCR-PC8E camcorders and stored on a computer.

Recordings from the two cameras overlooking the flight room were used to reconstruct the three-dimensional flight path of the bat and the positions of the microphones with the software Simi-Motion (Simi Reality Motion Systems GmbH, Version 6.5, Build 245). The software used a direct linear transformation algorithm to compute a 3-D position from the 2-D coordinates of the two video recordings.

A custom-built ultrasonic microphone (PC-Tape microphone, Animal Physiology, University of Tübingen, Germany) with a flat frequency response (±3 dB between 18 and 200 kHz) was positioned directly behind the landing grid, and recordings made with a sampling frequency of 480 kHz were synchronized with the video recordings. Control and synchronization of infra-red strobe, video and audio recordings was managed by PC-Tape (Animal Physiology, University of Tübingen, Germany).

The signals from the 16 microphones were amplified and then digitized with a sampling rate of 360 kHz and stored on a computer. For each approach flight, 4 s of audio and video recordings before landing were saved.

Synchronization of the video with the sound recordings was accomplished by comparing the sound pattern of three distinctive acoustic events on one of the 16 microphone recordings with the pattern of the PC-Tape microphone recording, which itself was synchronized with the video system through the VITC-code of the video cameras. The resulting synchronization accuracy was ±1 ms.

Data analysis

3-D movement analysis

Recorded videos were analyzed using the 3-D-Movement-Analysis software Simi-Motion (version 7.5.0.288). The beginning of the sequence was defined as the frame when bats were visible on the recordings of both cameras. The end of the sequence was the time of landing as determined with the third camera. For all recorded flights, the position of the head of the bat was determined on each half-frame (every 20 ms), and the resulting flight path was calculated. The reconstruction error was ±5 cm.

Sound analysis

Sound recordings were analyzed with a custom-made colour spectrograph using a 256-point FFT, a Hann-window and zero padding. The beginning and end of each call were defined at 6 dB below the maximum amplitude. The position of the bat at each call emission was interpolated from the position information available in intervals of 20 ms.

Computation of emission SPL

A custom-written Matlab (Version 7.0, The Mathworks, Natick, MA, USA) program called Sonarbeam was used to reconstruct the emission intensity at a distance of one meter (the reference distance) from a sound source (e.g. the mouth of a bat), depending on the direction and frequency band. A power spectral density (PSD, Welch's) was estimated over the time-window containing the call for each of the 16 microphone recordings. Each PSD was corrected for atmospheric and geometric attenuation by using the distance between the bat and each microphone, the directional frequency response of each microphone, as well as atmospheric parameters in order to obtain the emitted PSD one meter in front of the mouth of the bat in the direction of the microphones. From these resulting 16 PSDs, the emitted PSDs permeating a sphere of radius one meter around the source were interpolated on a fine spherical grid using cubic spline interpolation. The sound pressure level corresponding to the root mean square (r.m.s.) intensity was computed by integrating the PSD intensities for frequency bands between 20 and 110 kHz. The main axis or direction of the call was defined as the direction from the bat towards the highest interpolated SPL on the sphere. The source level refers to the SPL at one meter from the source in this direction. For an accurate interpolation of the maximum SPL of a call, the extent of the array must sample at least a large part of the central lobe, but the spatial sampling density of the microphones must be considerably tighter than a fraction of the width of the lobe. The minimum width of the emitted central lobe can be estimated from wave acoustics for a given maximum source size and emission frequency. The array dimensions used here are suited to study the SPL of bats, given the size of the mouth opening of the bat and using a piston model for a rough estimation of emission characteristics at 40 kHz.

Determination of the wingbeat cycle

By scoring the wing position from top (+5) to bottom (–5) in intervals of one, the position of the wing of the bat relative to its horizontal axis was determined for each frame until the wing position was not clearly visible [0.1–0.4 s before landing (Fig. 1)]. Using a frame rate of 50 Hz when recording a wingbeat at 10–15 Hz, we can reconstruct the wing movement without any aliasing effects. Cubic spline interpolation between scored wing positions was used to derive the wingbeat sequence (wing position over time). This wingbeat sequence was low-pass filtered using a Hanning window with a duration of slightly more than the wingbeat period in order

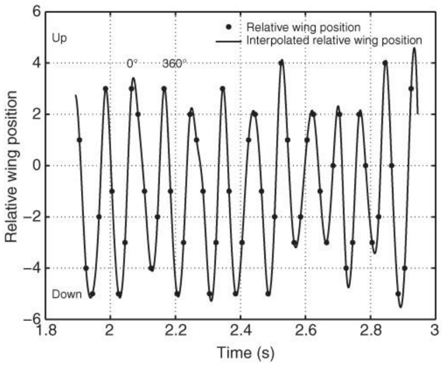

Fig. 1. Relative scored wing position and interpolated relative wing position for one flight. The maximal upward wing position is +5, maximal downward wing position is –5, horizontal wing position is 0. Wingbeat rate is relatively constant, with a cycle duration of 90 ms. Angular presentation in subsequent figures uses 0 deg and 360 deg as the topmost position of the interpolated wing position, and 180 deg as the undermost interpolated wing position.

to eliminate an offset due to unequal scoring (Fig. 4B). The residual between each wing position and the low-passed signal was used as the wing oscillation. This resulting oscillation was transformed through Hilbert transform into its corresponding analytic function. The angle of the analytic function was used as the phase of the wingbeat [0–360 deg (Fig. 1)].

Computation of the SL variation

Source levels for all calls emitted were high-pass filtered as a function of time to account for the individual-specific range-dependent SL reduction as the bat approached the landing site. In order to synchronize wing position with call emission, the distance between the bat and the landing site was used to compute and correct for the runtime of each call. Calls were categorized as single calls, groups of two (dyads) and groups of three (triplets). Calls emitted during the last part of the approach – that is, groups larger than three calls – were not analyzed. These calls were emitted when the bat was close to the array, where SL measurements were not very reliable. Here, the distance of a bat to the plane of the array is shorter than the distances between microphones, so the acuity of the interpolation process is insufficient. Additionally, the bats start to make the turn within this distance in order to land upside down. During this turn, some of the echolocation calls were directed away from the array. In addition the wingbeat was often not clearly visible 0.1 to 0.4 s before landing.

For each of the four bats, two flights were analyzed, resulting in 249 calls, of which 158 that were emitted as single calls (10), in dyads (73) or triplets (75) were considered for further analysis. Ninety-one calls were emitted in groups containing more than three calls and ignored in consequent analysis. Differences in the emission timing were tested using a Mann–Whitney *U* test with Holm–Bonferroni correction in SPSS 15 (Zar, 2009).

RESULTS

Timing of call emission relative to wing position

While approaching the landing site, the bats changed from single calls to groups with two, three or more signals. Call emission was synchronized with wing position. The emission of single calls, dyads or triplets was not evenly distributed over the entire wingbeat but showed a trimodal distribution, with one peak at the end of the downstroke, one peak during the upstroke when the wings were horizontal, and one peak at the topmost wing position (Fig. 2). Very few signals were produced in a range of a 60 deg width just before the middle of the downstroke. The centre of all call groups was positioned in the middle of the upstroke. Single calls and the second calls of triplets were therefore emitted at this wing position. Calls of dyads were emitted at the lowest wing position and just before the uppermost wing position, thus before and after the centre of the dyad. The first and the third calls of triplets were emitted earlier and later in the wingbeat, respectively; the first call at the end of the downbeat and the third call around the topmost position of the wing (Fig. 3). The group size-dependent timing of emission relative to the wingbeat cycle is significant. The first calls of triplets are emitted earlier than the first calls of dyads (P=0.003), which are emitted earlier than single calls (P<0.001). The same pattern can be seen for the second calls in each group [second of dyads and second of triplets, P<0.001 (Fig. 3)].

SL variation

The SL decreased while the bat approached the landing grid. This decrease was not steady, however, but showed a regular modulation when plotted over time (Fig. 4A). The maximum modulation within one period was 12 dB peak-to-peak (pp). The SL varied with the same frequency and phase as the wingbeat (Fig. 4C). Comparing the wing position with the SL modulation revealed an increasing positive SL deviation (from the moving average of the SL) during the upstroke, reaching maximal positive SL modulation just before the upper turning point of the wings. The average modulation was approximately 4 dB pp (Fig. 5). SL deviation decreased during the first part of the downstroke, and, during the second half of the downstroke, the average SL modulation reached a maximal negative modulation at 1.5 to 2 dB below the moving average (Fig. 5).

DISCUSSION

The aim of this study was to understand how variations of signal SL during the approach to a landing site correlate with and depend

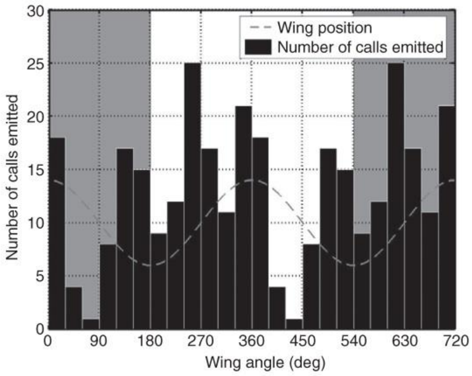

Fig. 2. Number of calls emitted during each 30 deg section of the wingbeat cycle (total number of calls considered: 158). Representation of two wingbeat cycles (grey dashed line) to illustrate circularity.

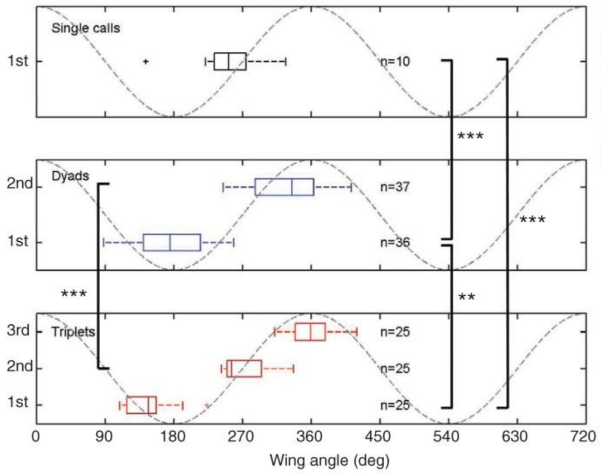

Fig. 3. Timing of call emission relative to wing-angle. The grey dashed line represents wing movement, incorporating two wingbeat cycles to illustrate circularity. Box plots show median, upper and lower quartile of the wing-angle at which calls were emitted for each call category. The number of calls included in each category is stated. The first calls of dyads are emitted significantly earlier in the wingbeat cycle than single calls. The first calls of triplets are emitted significantly earlier than first calls of dyads. Second calls of triplets are emitted significantly earlier than second calls of dyads.

on wingbeat and respiratory cycle. In flying bats, sound emission is often correlated with wingbeat and respiratory cycle. Therefore we will use the correlation between sound emission pattern and wingbeat as an indirect indicator for the correlation between respiratory cycle and wingbeat. We will then discuss the variations in signal SL in relation to the presumed respiratory cycle and hypothesize about the influence of muscle movements during each wingbeat cycle on the source level. Finally, we will discuss how group size correlates with the timing of call emission in relation to the wing position.

Respiratory cycle as indicated by the correlation between sound emission and wingbeat cycle

When approaching a target, *E. fuscus* group their echolocation calls into dyads, triplets and larger groups. All groups and also single calls emitted are centred at about the middle of the upstroke. The pauses between groups are centred just before the middle of the downstroke. Grinnell and Griffin (Grinnell and Griffin, 1958) first proposed that the grouping of calls, already apparent in the earliest recordings of bat echolocation signals (Galambos and Griffin, 1942), reflect the respiratory cycle. Recordings of diaphragmatic

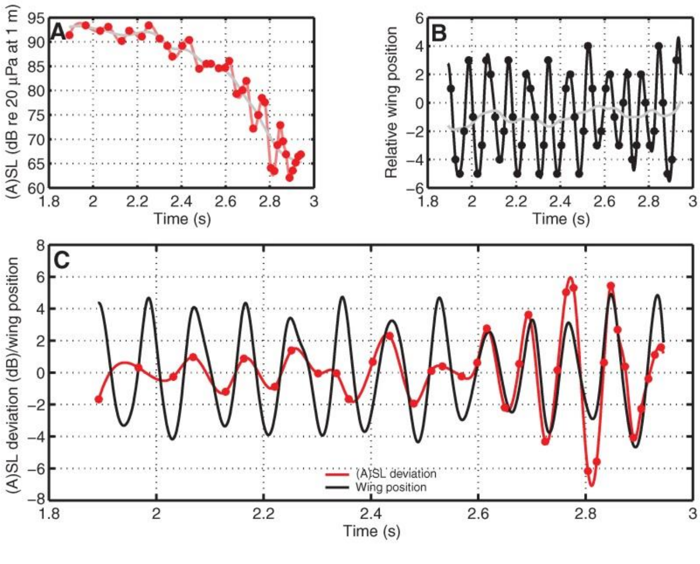

Fig. 4. (A) Source level (SL; ASL for the first calls) within 1.2 s while one bat approaches the landing grid. Red dots indicate (A)SL for each call, the red line interpolated (A)SL, and the grey line the low-pass-filtered (A)SL. (B) The relative scored wing position and interpolated relative wing position for same flight. The maximal upward wing position is +5, the maximal downward wing position is –5, and horizontal wings are 0. The grey line is the low-pass-filtered wing position. (C) Overlaid (A)SL variation and wingbeat.

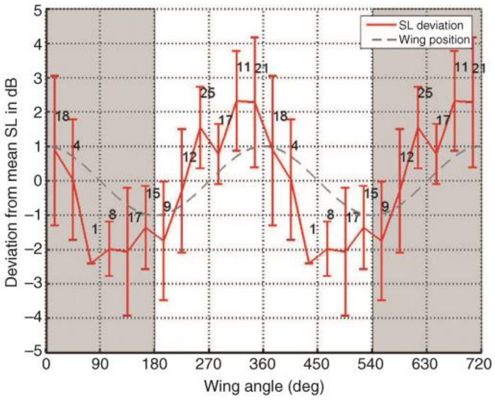

Fig. 5. Mean (A)SL modulation (±s.d.) relative to wing position for each 30 deg section of the wingbeat cycle. Representation of two wingbeat cycles (grey dashed line) to illustrate circularity. The sample sizes of each 30 deg section are given next to the error bars.

myopotentials showed that inhalation in flying *P. parnellii* occurred during the pauses between call emissions (Lancaster et al., 1995). Other experiments confirmed that calls are emitted during exhalation and that pauses between groups are due to inhalation in stationary horseshoe bats (Schnitzler, 1968) and for flying *Phyllostomus hastatus* (Suthers et al., 1972). We therefore conclude that *E. fuscus* approaching a landing platform inhaled in the pauses between groups just before the middle of the downstroke and exhaled with the potential to emit signals from the end of the downstroke throughout the upstroke until the first part of the downstroke. Correlation patterns between wingbeat and call emission found in most previous studies are similar to our pattern – that is, the pauses are positioned in the downbeat, thus indicating the inspiration phase (Schnitzler, 1971; Suthers et al., 1972; Schnitzler and Henson, 1980; Schnitzler et al., 1987; Kalko and Schnitzler, 1989; Wong and Waters, 2001; Wilson and Moss, 2003; Parsons et al., 2010). There are, however, some exceptions. Kalko (Kalko, 1994), studying free-flying pipistrelle bats, describes a wingbeat pattern where most of the signals were emitted during the downbeat. Schnitzler and Henson (Schnitzler and Henson, 1980) described that bats intercepting an insect make several wingbeats while emitting a long terminal group. An example of this can be seen in Fig. 4C, where the bat emits a series of 11 calls at a pulse interval of 11–16 ms during the last 1.5 wingbeats shown. Inspiration in the terminal group will most likely be omitted for one or more wingbeats. Moss and colleagues (Moss et al., 2006) found that *E. fuscus* sometimes overrode the tight coupling of wingbeat and call emission. In conjunction with landing, obstacle avoidance or take-off, call production was shown to occur during any point of the wingbeat cycle (Lancaster et al., 1995). This indicates that bats can voluntarily break the strict coupling of wingbeat, respiratory cycle and emission of either single signals or signal groups. The bats in this study, however, were trained to make stereotyped approach flights and did not break this tendency when emitting single calls, dyads or triplets.

Correlation between SL variation, wingbeat and subglottic pressure

The SL decrease during the approach to a landing platform was superimposed by regular wingbeat-cycle-coupled oscillations. During the upstroke, the SL was raised in relation to the moving average. The maximal positive SL modulation was reached just before the uppermost turning point of the wings. Calls emitted at the uppermost turning point were on average already more than 1 dB less intense. Calls emitted around the lowermost turning point of the wings were on average 4 dB lower in SL than calls emitted just before the uppermost turning point. Previous studies did not describe a comparable modulation, possibly owing to methodological limitations. There are some indications that other species also modulate the SL in the rhythm of the wingbeat. Parsons and colleagues (Parsons et al., 2010) found that calls emitted during the upstroke of *Mystacina tuberculata* were on average 1.5 dB more intense than during the downstroke. However, this difference was not significant and was not discussed further. Using only one microphone, modulations could also be caused by scanning movements of the bat. By using a 16-microphone array, we measured the SL and the modulation thereof accurately and showed that the variations are not caused by head movements. Recordings of *Myotis daubentonii* using one microphone also indicate a modulation of the SL in the rhythm of the wingbeat [fig. 3A in Boonman and Jones (Boonman and Jones, 2002)].

The SL variation is most likely caused by changes in the subglottic pressure during the wingbeat cycle. Subglottic pressure increases immediately before vocalization and is positively correlated with the SPL of the echolocation calls (Fattu and Suthers, 1981). Abdominal wall muscles generate the primary power for vocalization, and there is little difference in abdominal wall muscle activity between vocalizing bats at rest and during flight (Lancaster et al., 1995; Lancaster and Speakman, 2001). Lancaster and colleagues (Lancaster et al., 1995) proposed that the coincident contraction of the flight muscles *pectoralis* and *serratus ventralis* during flight could cooperate in the pressurization of the thoraco-abdominal cavity. This increase of pressure in the thoraco-abdominal cavity could lead to an increase of the subglottic pressure and thus provide the precondition for echolocation calls with a slightly higher SL. The SL increase relative to the average in the second half of the upstroke coincides with the onset of the flight muscle contraction (Hermanson and Altenbach, 1981; Hermanson and Altenbach, 1983). Flight muscle contraction terminates during the first half of the downstroke, which could cause the reduction of the SL relative to the average. Inhalation then takes place after the major wing muscles have ceased activity (Lancaster et al., 1995). To validate this hypothesis, accurate measurements of the SL of free-flying bats in combination with electromyographic recordings of the major flight muscles are necessary.

Theoretically, the described dependency of signal SL on wing position would give bats the possibility of changing the SL of single signals by emitting them at different phases of the upstroke. When signals with a high SL are needed – that is, when searching for prey – calls should be emitted at the end of the upstroke. This could explain why bats searching for prey in the field emit loud echolocation calls when the wings are near the topmost position (Schnitzler et al., 1987; Kalko and Schnitzler, 1989). Whether bats actually use this possibility must be investigated in further studies.

The strong variation of SL in correlation with wingbeat challenges the intensity compensation theory, according to which a reduction of the SL in bats closing in on a target compensates for the increase of the received echo level at the ear of the bat owing to a reduction of transmission loss and keeps the echo level constant (Kobler et al., 1985; Hartley et al., 1989). During an approach, the SL is reduced by approximately 30 dB over a distance of about 2.0–1.5 m. However, if the SL fluctuates by a maximum of 12 dB (on average about 4 dB) within a single wingbeat, one can hardly speak about a constant received level available for the bat. This supports the hypothesis that the average reduction of the SL during an approach by approximately 6 dB per halving of distance is not made in a tightly coupled feedback control system (J.C.K., unpublished observations).

Correlation of group size and call emission timing

Single calls were emitted relatively late in the wingbeat cycle – during the upstroke. The first calls of dyads were emitted earlier, starting at the end of the downstroke, and second calls of dyads were made mainly during the second half of the upstroke. In triplets, this pattern continued: first calls were again emitted earlier, already during the downstroke, second calls during the upstroke and third calls during the upper turning point of the wings. The temporal patterning of the calls relative to the wing position was changed so that the centre of the group relative to the wingbeat remained constant during the upstroke when the wings were approximately horizontal. When group size was increased, first calls were emitted earlier, last calls slightly later, and the variability tended to decrease.

This indicates that the number of calls a bat is going to emit in the upcoming group is indicated by the timing of the first call relative to the wing position when the bat produces single pulses, dyads and triplets in a stereotyped pattern. This suggests that the emission of pulse groups follows a fixed motor pattern, where the information gained with the preceding pulse group determines how many calls will be emitted in the next group. This might be the reason why pulse groups are separated by rather long 'in between group pulse intervals', which should be in the range of the vocal reaction time to new auditory information.

LIST OF ABBREVIATIONS

ASL	apparent source level
PSD	power spectral density
r.m.s.	root mean square
SL	source level
SPL	sound pressure level

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5.2 Scanning behavior in echolocating common pipistrelle bats (*Pipistrellus pipistrellus*)

This paper is part of Anna-Maria Seibert's doctorate thesis. I assisted in array design and in the development of the recording software, I adapted the employed localization software, provided additional matlab programs, discussed the data and results and commented on the manuscript.

Citation:

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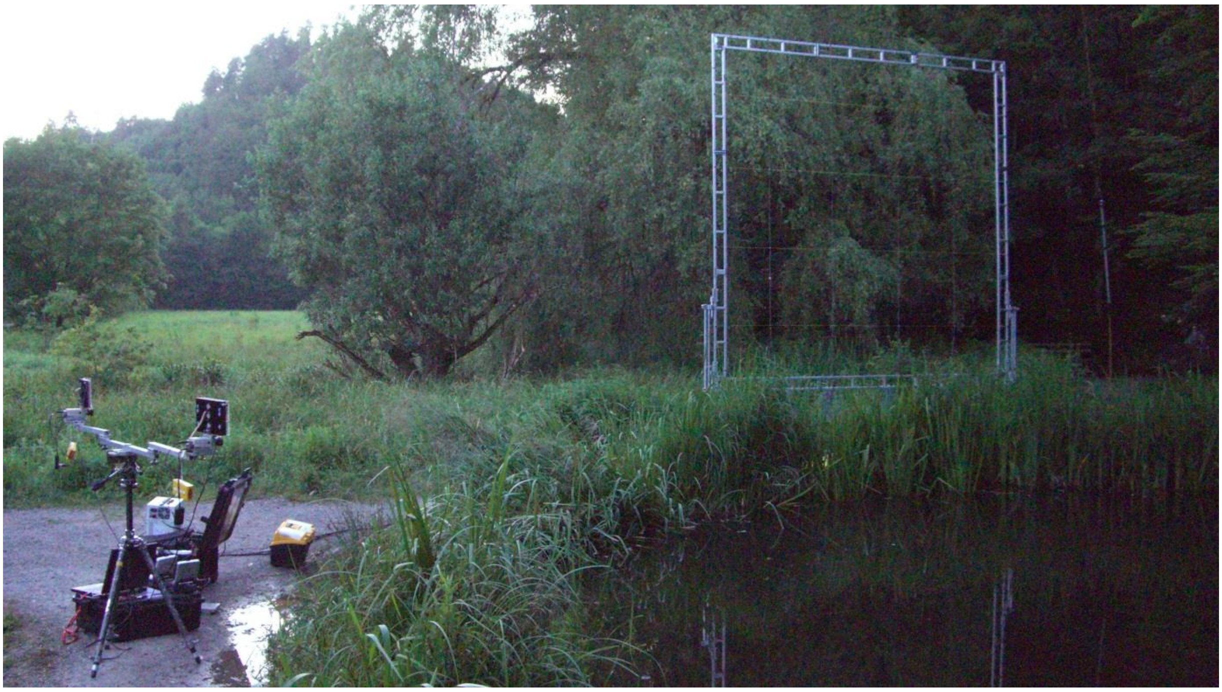

Figure 16: The planar 16 microphone array and the stereo IR video system set up at a pond in Bebenhausen. Photo: Jens Koblitz

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Scanning Behavior in Echolocating Common Pipistrelle Bats (*Pipistrellus pipistrellus*)

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Abstract

Echolocating bats construct an auditory world sequentially by analyzing successive pulse-echo pairs. Many other mammals rely upon a visual world, acquired by sequential foveal fixations connected by visual gaze saccades. We investigated the scanning behavior of bats and compared it to visual scanning. We assumed that each pulse-echo pair evaluation corresponds to a foveal fixation and that sonar beam movements between pulses can be seen as acoustic gaze saccades. We used a two-dimensional 16 microphone array to determine the sonar beam direction of succeeding pulses and to characterize the three dimensional scanning behavior in the common pipistrelle bat (*Pipistrellus pipistrellus)* flying in the field. We also used variations of signal amplitude of single microphone recordings as indicator for scanning behavior in open space. We analyzed 33 flight sequences containing more than 700 echolocation calls to determine bat positions, source levels, and beam aiming. When searching for prey and orienting in space, bats moved their sonar beam in all directions, often alternately back and forth. They also produced sequences with irregular or no scanning movements. When approaching the array, the scanning movements were much smaller and the beam was moved over the array in small steps. Differences in the scanning pattern at various recording sites indicated that the scanning behavior depended on the echolocation task that was being performed. The scanning angles varied over a wide range and were often larger than the maximum angle measurable by our array. We found that echolocating bats use a "saccade and fixate" strategy similar to vision. Through the use of scanning movements, bats are capable of finding and exploring targets in a wide search cone centered along flight direction.

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Introduction

Bats use echolocation for spatial orientation and foraging. Their echolocation signals are emitted through either the mouth or nostrils, and form a directional sonar beam [1,2]. The aiming of this sonar beam is determined by the head position during sound emission. The directional beam covers only a partial field of the world around a bat. It has been estimated that bats can react to prey flying within a "search cone" which is up to 120–150° wide [3,4]. However, it is not known how head and beam are oriented when bats perform specific echolocation tasks in the field. Do bats always direct head and beam in flight direction so that the directionality of the beam alone determines the search cone or do they probe the environment with scanning movements, thereby increasing search volume?

To address these questions it is necessary to determine the sonar beam aiming in free- flying bats. This is only possible if the flight path of a bat is known and their signals are sampled with sufficient spatial resolution such that the reconstruction of the beam is possible and that its aiming direction relative to the bat's flight path can be determined.

The possibility of bats actively changing their sonar beam aiming has been derived from single microphone recordings in the field. Successive signals often show distinct changes in signal amplitude. These could be indicators for scanning movements [4,5,6,7]. However, this periodic variation in bats has been argued to be due to deliberate amplitude variation as a strategy for correct pulse-echo pair assignation [8]. In one of the few studies examining beam-aiming behavior in the field, three stationary microphones were used to conclude that *Eptesicus serotinus* point their beam downward when flying high and forward when flying at lower altitudes [9].

In the laboratory, scanning studies have been made with linear microphone arrays where trained bats directly approached prey [10,11,12,13] or after flying through a gap [14]. The array used in these experiments consisted of microphones arranged on a plane, in a U-shaped arrangement along three walls of a flight room. The reconstructed beams were therefore only horizontal cross sections of the beam, thus the observed scanning movements described only the horizontal movement of an apparent beam maximum. Vertical movements were not indicated. Nevertheless, the experiments provided interesting results on the scanning behavior of bats. When bats were trained to fly through a gap *Eptesicus fuscus* displayed sequential scanning of the two edges of the opening [14]. When bats searched for prey in the laboratory, scanning movements in the horizontal plane were also described but not quantified [10]. During the approach to prey *E. fuscus* directed the apparent beam maximum on the prey. This led to the general conclusion that bats lock their real beam maximum onto a single target of interest while approaching it. More recently, a two-dimensional 16 microphone array was used to determine the sonar beam aiming in landing. *E. fuscus*. The bats attended to the target of interest by keeping the sonar beam locked onto the landing site during the approach beginning at distances of 1–2 m [15].

From analyses from head aiming in photographs or videos, it was also concluded that bats direct their sonar beam towards a target when approaching it [16,17] or follow moving targets with the beam while sitting on a platform [18].

Sequential acquisition of sensory information by the scanning of an environment with a sensory organ has been studied mainly in the visual systems of humans and animals. Humans look at or search for objects by integrating head and eye movements to visual gaze saccades [19]. After each saccade they fixate a new area of interest and retain the visual image on the fovea. Such foveal fixations take 200–300 ms on average [20]. The gaze saccades that connect such fixations are task-specific, and depend on the observer's behavioral goals [21].

Ghose and Moss (2003) made an analogy to visual gaze by terming the bats' aiming with their sonar beam as an "acoustic gaze". Surlykke et al. (2009) compared the scanning behavior in bats to active visual scanning. We also hypothesize that echolocating bats use a sequential "fixate and saccade" strategy comparable to visual systems [22]. To acquire information on the acoustic world around them, bats are able to move their acoustic gaze from one pulse or fixation to the next. Bats continuously emit echolocation signals and the analysis of each pulse-echo train delivers information comparable to the visual information delivered by each foveal fixation. By shortening the interval between pulses, bats can increase the rate of fixations and with it the update rate of new information.

In this work we were interested in the scanning behavior of pipistrelle bats (*Pipistrellus pipistrellus*) flying in the field in open and edge space. Our hypothesis is that bats in the field make scanning movements, or "acoustic gaze saccades", which are comparable to visual scanning. We assume that their scanning behavior is also task specific. We therefore determined the scanning behavior of bats in relation to performing various tasks including searching for prey, orienting in space, and approaching an obstacle at three different recording sites (referred to as forest, farm, and garden).

Results

Flight and Echolocation Behavior

In edge space, the reconstructed flight paths of *P. pipistrellus* flying towards the microphone array and the corresponding sound sequences allowed for a discrimination of two distinct behaviors at all three recording sites. Beyond a ~2 m distance to the array, bats exhibited flight directly towards the microphone array with roughly constant flight speed (Figure 1B–E). Search calls with interspersed hunting sequences indicated that the bats were foraging. From these results, we concluded that the bats were in search flight and that their behavior corresponded to search behavior. At ~2 m in front of the array, the bats switched from search to approach behavior. During approach, bats made avoiding maneuvers resulting in passes around or through the array (Figure 1B–E). Signal parameters during approach were characterized by higher bandwidth, shorter duration, shorter pulse intervals, lower terminal frequency, higher pulse density, and reduction in source level (SL) (Figure 2). In open space, bats were foraging at heights several meters above ground level. As these recordings were obtained with a single microphone we could not determine exact locations and flight paths of individual bats.

Search flight in edge and open space. In edge space, the bats' behavior during search flight differed between the three recording sites. In the forest, individuals flew nearly straight at heights between 0.5–4.5 m and directly towards the microphone array (Figure 1C). Average flight speed was 5.4 m/s. Mean call duration was 3.1±0.7 ms at a mean pulse interval of 91.2±7.5 ms. Terminal frequency of calls was 48.0±1.3 kHz, and the source level was 84.5±2.1 dB SPL *re* 20 µPa at 1 m (Table 1). On the farm, individuals flew around a corner and passed along a house wall at a close distance and at heights between 1–3 m above ground (Figure 1D). Bats were also observed circling, presumably in search of prey. Mean call duration was 3.0±0.6 ms with a pulse interval of 89.6±4.3 ms. Terminal frequency was 46.5±3.4 kHz, and the source level was 93.8±5.0 dB (Table 1). In the garden, the bats were usually foraging, and would circle at heights of between 1–3 m above ground in front of the microphones (Figure 1E). Mean call duration was 3.8±1.0 ms at a mean pulse interval of 86.4±3.3 ms. Terminal frequency was 48.4±1.3 kHz, and the source level was 95.8±2.1 dB SPL (Table 1). Different recording localities compared to each other showed a significant difference in mean SPL with the forest site with 85 dB having the lowest SPL of all three recording sites (Table 1., $F_{2,29} = 46.00$, $p < 0.0001$; Tukey HSD, $p \leq 0.05$).

In open space, where bats do not react to the background in their echolocation behavior [23], a mean signal duration of 6.2±0.8 ms was significantly longer than the duration in the three edge space situations ($F_{3,50} = 64.14$, $p < 0.0001$; Tukey HSD, $p \leq 0.05$). The pulse interval with 97.7±4.7 ms was also significantly longer than in the other three locations ($F_{3,49} = 11.24$, $p < 0.0001$; Tukey HSD, $p \leq 0.05$) (Table 1). Recordings with one microphone did not allow for a determination of the SL.

Approach flight in edge space. The behavior of bats during approach flights at the three recording sites was similar to that previously described for pipistrelle bats [2]. In the forest, most bats avoided the array and flew around it, while only one flew through the array. At the farm no bats passed through the microphone array. They mostly passed above or through the gap between the array and the house wall. Only a few flew around the array on the right side. During approach the bats usually kept a speed of 4–6 m/s. However, when bats made sharp avoiding maneuvers or passed between the microphones, flight speed was reduced to approx. 2.5 m/s. At the garden site, bats rarely approached the array.

Signal parameters during approach stereotypically changed to a shorter duration, shorter pulse intervals, and a reduction in SL. Call duration and pulse interval were reduced at the closest point in front of the array to ~ 1 ms and 50 ms, respectively. In halving the distance to the array, the SL was reduced by ~ 8 dB SPL ($n = 182$; $y = 25*\log*(\text{distance})+75$; $R^2 = 0.45$).

Scanning Behavior in Search Flight

Reconstructed flights with aiming vectors (Figure 1) describe the scanning behavior of bats while flying towards the microphone array. Bats often moved their beam back and forth within a search cone pointing in flight direction. The scan paths of the apparent and real beam maxima on the array plane also characterize the scanning behavior (Figure 3). The comparison of the scan paths at the three recording sites revealed distinct site-dependent differences. The differences in the patterns of the angular orientation of the beam movements indicate that the scanning behavior was site- and therefore task-specific (Figure 4A–C). On the forest road, scanning movements were mostly diagonal between the upper right corner of the

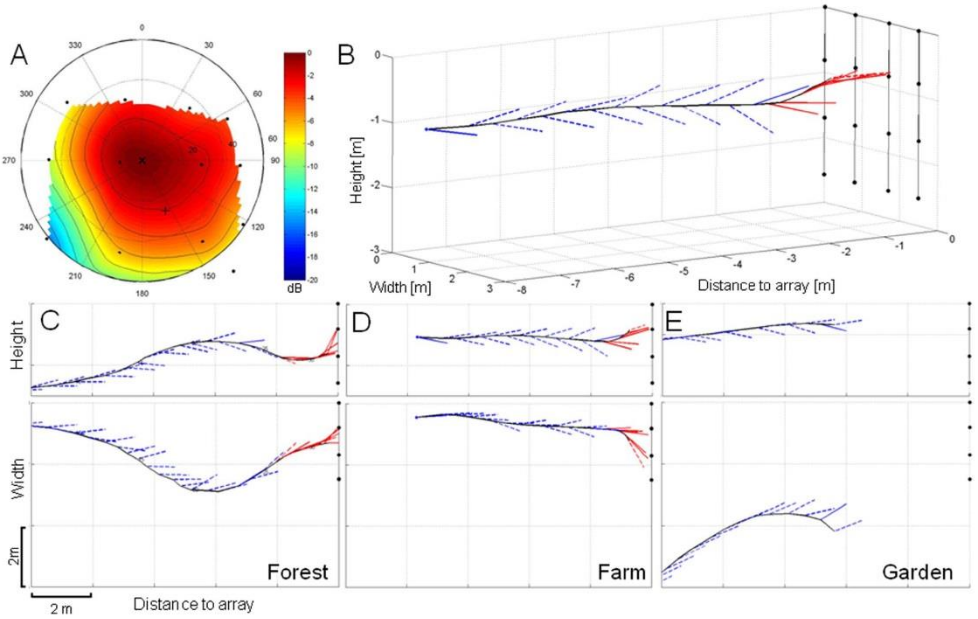

Figure 1. Beam reconstruction and exemplary flight paths towards the array at different edge habitat recording sites. Reconstructed sonar beam (A) and three-dimensional display of a flight towards the microphone array (B). The SPL in (A) is color-coded and indicates the beam form relative to the beam maximum in the center of a polar plot. The black dots mark the positions of the microphones, the cross representing the flight direction. C–E depict side views (upper row) and overhead views (lower row) of exemplary flights towards the microphone array which was positioned ~ 1.6 m above ground at the three recording sites: (C) forest road, (D) farm, (E) garden. The flight paths are depicted as black lines. Each of the blue (search calls) and red (approach calls) vectors begins at the bat's position at the time of call emission and points towards the calculated position of the reconstructed apparent (dotted line) or real (solid line) beam maximum on the array plane. The black dots represent the 16 microphones of the array. **Note:** the apparent vectors do not indicate the real aiming of the beam, and the angle between successive vectors not the real scanning angle. The real scanning angles may be much larger than the apparent scanning angles on this graph. The real scanning angle is only indicated if the beam maxima were within the array.
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array and the lower left corner. Scanning movements up and down occurred less often, with movements to the left and right side occurring the least (Figure 4A). At the farm, scanning movements up and down were dominant, followed by diagonal scanning movements. Scans to the sides were rarely performed (Figure 4B). At the garden, scanning movements to the left and right dominated, but movements up, down, and diagonal also occurred (Figure 4C). In search flight, pipistrelle bats often scanned at angles larger than those that could be observed by our array, resulting in apparent scanning angles (Figure 5A). The largest apparent angle measured was 51°, and the largest real angle was 42°. Successive calls sometimes pointed in the same direction, and are therefore assigned a scanning angle of 0°.

The single microphone recordings from open space revealed clear amplitude differences in successive calls in 90% of the recordings, totaling more than 200 calls (18 of 20 sequences, Figure 6B). The mean difference was 8.1±5.3 dB, but differences ranged as high as 24 dB. When examining recordings of a single microphone in the array, we also found clear and often alternating variations of amplitude of successive search signals in 96% of sequences from the three sites in edge space (76 of 79 sequences, Figure 6A). The mean difference of consecutive calls was 6.7±3.7 dB, with a maximum difference of 23 dB. These distinct variations in search flight can be explained by the documented scanning movements, likely due to the fact that a single microphone picked up different parts of the sonar beam of successive pulses. In both situations, we found distinct alternating changes of signal SPL (Figure 6C for edge space and (E) for open space) and less obvious, less regular changes in signal SPL among succeeding calls (Figure 6D for edge space and (F) for open space).

Scanning Behavior in Approach Flight

During approach the scan path of the real beam maximum remained mainly within the array plane, e.g., the scan path moved around the inner four microphones forming the gap which was passed by an individual (Figure 3A center plot) or the scan path moved towards the right side as an individual passed the array (Figure 3B upper plot).

In the forest, the angular direction of most scan paths pointed upwards (Figure 4D). Here, bats often passed the array by flying over it. At the farm, the scan path angles pointed more to the right side where most of the bats passed (Figure 4E). At the

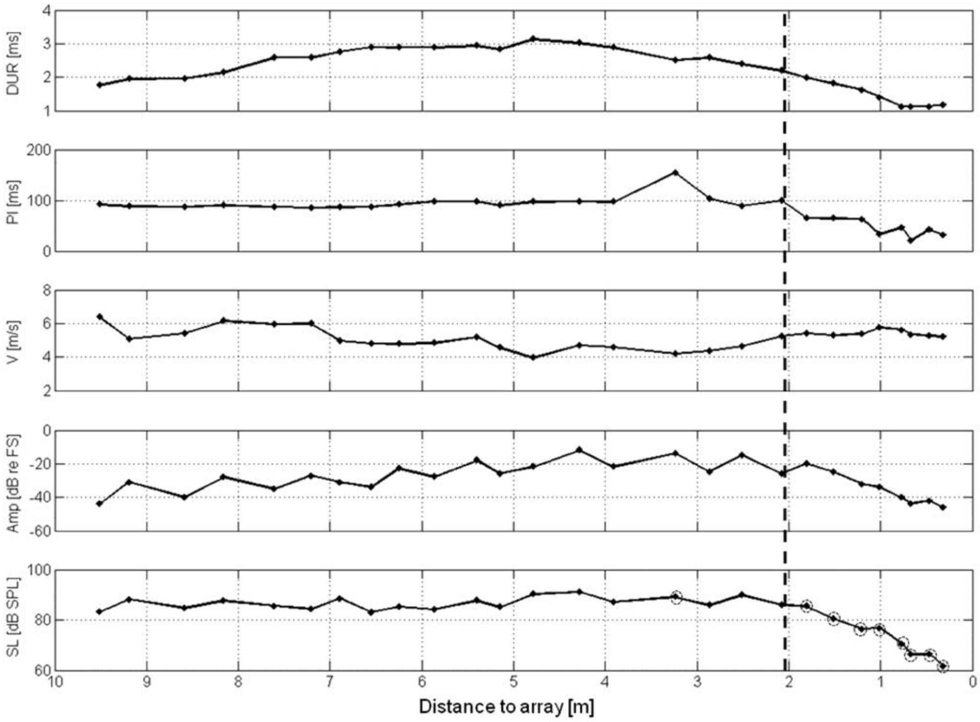

Figure 2. Echolocation behavior and flight speed of a *P. pipistrellus* flying towards the microphone array. The parameters pulse duration (DUR), pulse interval (PI), flight speed (V), max. signal SPL measured at the upper right microphone of the array (amplitude in dB relative to full scale), and source level (SL in dB SPL *re* 20 µPa at 1 m) of one typical flight plotted over distance to the array. In the lowest graph, real source levels (SL) of signals within the array are marked by circled points, all other values indicate apparent source levels (ASL). The dashed line indicates the beginning of the approach.
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garden, we did not obtain any approach calls as the bats did not pass the array. Closer than ~ 2 m to the array, most real scanning angles were below 10° (Figure 5A and B), thus the scanning movements during approach were smaller than in search flight.

Discussion

To our knowledge, this study is the first to describe the scanning behavior of an echolocating bat species (*P. pipistrellus*) when flying under natural conditions in the field. Using a planar 16-microphone array allowed us to not only reconstruct flight paths and assign source levels, but also to reconstruct the real aiming direction of the sonar beam (provided its maximum was within the array). Often the maxima of the recorded signals were not within the array such that it was only possible to reconstruct horizontal or vertical cross sections of the beam along the row of microphones with the apparent beam maximum. The scan path of apparent beam maxima also revealed how bats moved the beam and how these movements were influenced by the echolocation tasks the bats performed.

Flight and Echolocation Behavior during Different Echolocation Tasks

The echolocation tasks of bats depend on where they search, find, and acquire food. These tasks differ depending on the habitat type and the foraging situation.

Open space is defined as the foraging habitat where bats do not react to background targets [23]. We assume this lack of reaction indicates that spatial orientation in relation to the ground is not an important echolocation task, and that the bats mainly search for insects and increase their chances to detect prey by choosing suitable signals from their signal repertoire. The distinctly longer pulse intervals and signal durations in our single microphone recordings indicate that the bats were foraging in open space. The observed echolocation behavior was similar to that reported for bats foraging in the open [24].

In edge space, bats adjust their echolocation behavior to the background echoes by shortening the pulse duration, increasing the bandwidth, and reducing the SPL of their signals with decreasing distance to the background [1,4,17,25]. When foraging in edge situations bats have to simultaneously perform two different tasks. They perform spatial orientation tasks while

Table 1. Parameters of pipistrelle bat search signals.

		Forest	Farm	Garden	Open Space
		($n = 183$, $N = 15$)	($n = 82$, $N = 8$)	($n = 137$, $N = 9$)	($n = 225$, $N = 22$)
Duration [ms]	**mean** (±SD)	**3.1** (±0.7)	**3.0** (±0.6)	**3.8** (±1.0)	**6.2** (±0.8)
	min	2,3	2,1	2,0	4,0
	max	4,5	3,8	4,7	7,5
		($n = 139$, $N = 15$)	($n = 67$, $N = 8$)	($n = 107$, $N = 9$)	($n = 102$, $N = 21$)
Pulse interval [ms]	**mean** (±SD)	**91.2** (±7.5)	**89.6** (±4.3)	**86.4** (±3.3)	**97.7** (±4.7)
	min	80,6	85,2	81,6	89,5
	max	102,6	97,3	92,6	104,6
		($n = 183$, $N = 15$)	($n = 82$, $N = 8$)	($n = 137$, $N = 9$)	($n = 225$, $N = 22$)
Terminal frequency [kHz]	**mean** (±SD)	**48.0** (±1.3)	**46.5** (±3.4)	**48.4** (±1.3)	**45.3** (±1.6)
	min	45,5	38,7	47,0	43,2
	max	50,2	49,0	51,5	49,1
		($n = 183$, $N = 15$)	($n = 82$, $N = 8$)	($n = 137$, $N = 9$)	
Source level [dB SPL]	**mean** (±SD)	**84.5** (±2.1)	**93.8** (±5.0)	**95.8** (±2.1)	N/A
	min	81,0	84,7	90,9	N/A
	max	87,5	98,8	97,9	N/A

Parameters of search signals in *Pipistrellus pipistrellus* emitted when flying towards the array at three edge habitat recording sites and in open space. For each sequence only the mean of the contained parameters were used for statistics to reduce pseudoreplication. In edge space recordings, only calls within a distance of 3–10 m from the array were analyzed, so as to exclude approach signals. n = number of calls used to calculate the corresponding mean. N = number of recorded sequences.

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navigating along the background targets and reacting to unknown obstacles such as our microphone array. Additionally, they search for flying insects, a task that may be influenced by clutter echoes from the background. The observed echolocation behavior in our study was similar to that reported for bats foraging in edge situations [24]. However, behavior differed between the three recording sites, which may reflect differences in the spatial orientation task, e.g., differences were found in the source levels. In more open edge habitats including the garden and farm sites, bats emitted signals averaging 96.1 and 95.4 dB, respectively. In the more closed gap situation at the forest site, signals only reached an average of 84.8 dB. We assume that the bats lowered their calling amplitudes to reduce background echoes in the more closed situation, whereas in the more open situations at the farm and the garden, the bats use higher SLs. A similar explanation for the lowering of the SLs of *Macrophyllum macrophyllum* was used when flying near vegetation [26]. Site-dependent differences in SL were also found and explained by different foraging situations or individual differences [8].

When approaching the microphone array, bats had to perform an additional spatial orientation task. They had to collect all of the information necessary to guide their flight path around or through the unknown obstacle. The switch to approach behavior found in our study is in accordance with earlier findings in pipistrelle bats that react to prey or targets at a distance of ~ 2 m [17,24]. During the approach to the array, flight speed was reduced and signal duration and pulse interval decreased in a stereotypical manner, while SL was reduced by an average of 8 dB per halving of distance. This is slightly higher than the previously reported 6 dB per halving of distance to the target [27,28]. However, [15] also reported SPL reductions of 9 dB per halving of distance in landing *E. fuscus*.

Scanning Behavior during Different Echolocation Tasks

We found that pipistrelle bats often scan their environment by changing the sonar beam direction from pulse to pulse, most likely by rapid head movements. The beam can be aimed in all directions within a rather wide cone around the flight direction and it is not only moved in the horizontal plane, as indicated by recordings of the apparent beam maxima with horizontal microphone arrays [10,11,13,14]. The scanning patterns ranged from almost no beam movement to very large scanning movements between successive pulses. The largest real scanning angle we could measure with our array was 42°. Many calls had maxima outside our array, indicating that even larger angles were possible.

Sequential scanning behavior of a sensory system is best understood in vision. The aiming of the sonar beam can be compared to a visual gaze. Therefore we also use the term "acoustic gaze" [10]. The visual gaze system produces head and eye movements, or gaze saccades [19], so that the object of interest can be examined with the fovea. Visual information is sequentially acquired. Once a target of interest is found, the gaze attends to it and explores it with a sequence of saccades and foveal fixations. In humans, single visual fixations can take 100–600 ms [29], thus indicating rather big differences in the rate at which input of sensory information is updated in the sequential visual process.

Bats construct their auditory world by also utilizing a sequence of beam movements and fixations similar to the "fixate and saccade" strategy in vision [22]. Sequential sampling of two edges of a gap has been already demonstrated by Surlykke et al. (2009), who trained *E. fuscus* to fly through an opening in a fine net and measured the apparent beam direction in the horizontal plane. The information contained in each pulse-echo train can be compared to the information gained by each foveal fixation in the visual process. One difference between these systems lies in the speed at which updates can occur; the updating rate for succeeding acoustic fixations in bats is much faster than in the human visual system. When searching for prey in open space, and when

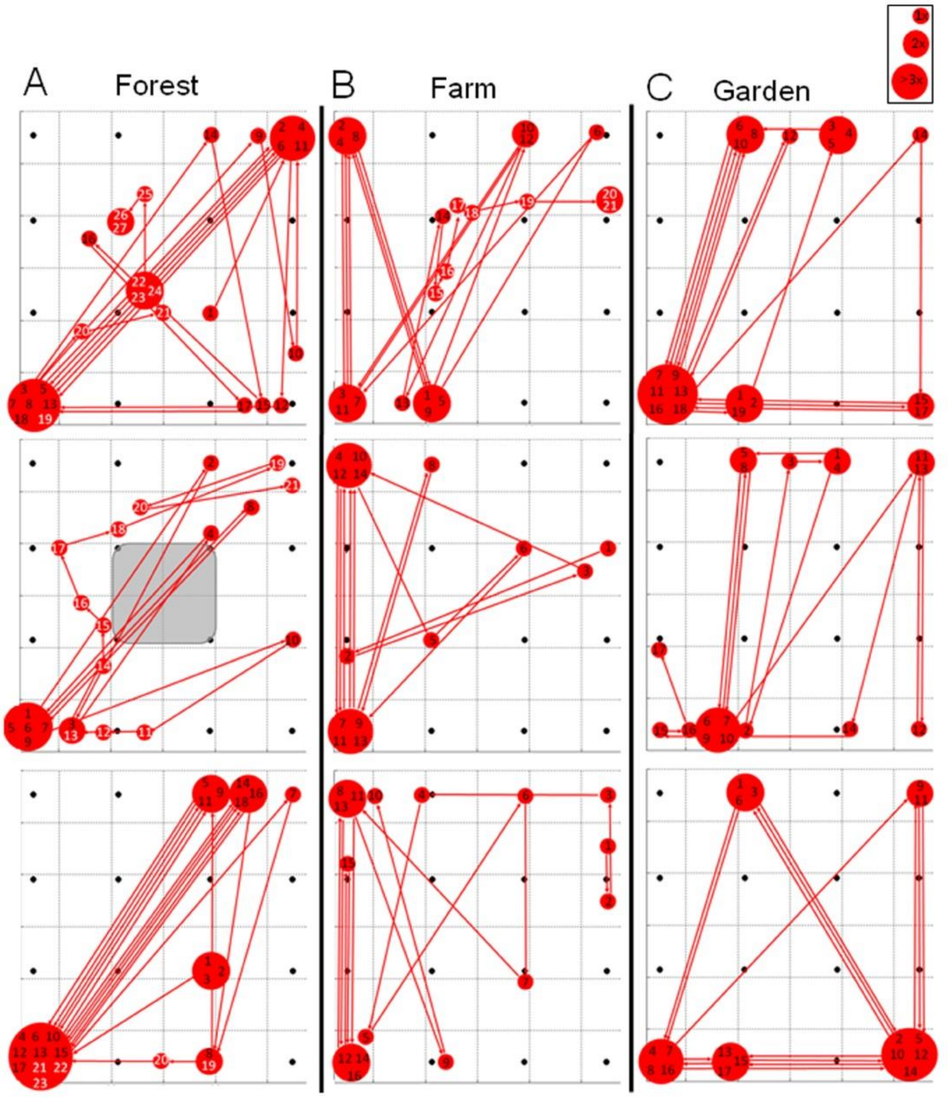

Figure 3. Scan paths at three edge habitat recording sites. Scanning behavior during three typical flights at each of the three edge habitat recording sites. (A) forest road, (B) farm, (C) garden. The scanning behavior is indicated as pulse-to-pulse scan path of the calculated apparent or real beam maximum on the array plane. Successive pulses are identified by either black (search) or white (approach) numbers. The larger the circle around the numbers, the more calls were pointed at this spot. All beam maxima pictured on the outer edges of the array are apparent beam maxima; real maxima are within the array. The grey square at the forest site depicts the location where a single bat flew through the array.
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navigating along edges, bats produced signals with intervals around 90–100 ms, corresponding to approximately 10 updates per second. During the approach of the array pulse intervals were even shorter, and similar to an approach towards a net opening, a landing site, or prey [10,14,15]. In humans the updating rate is

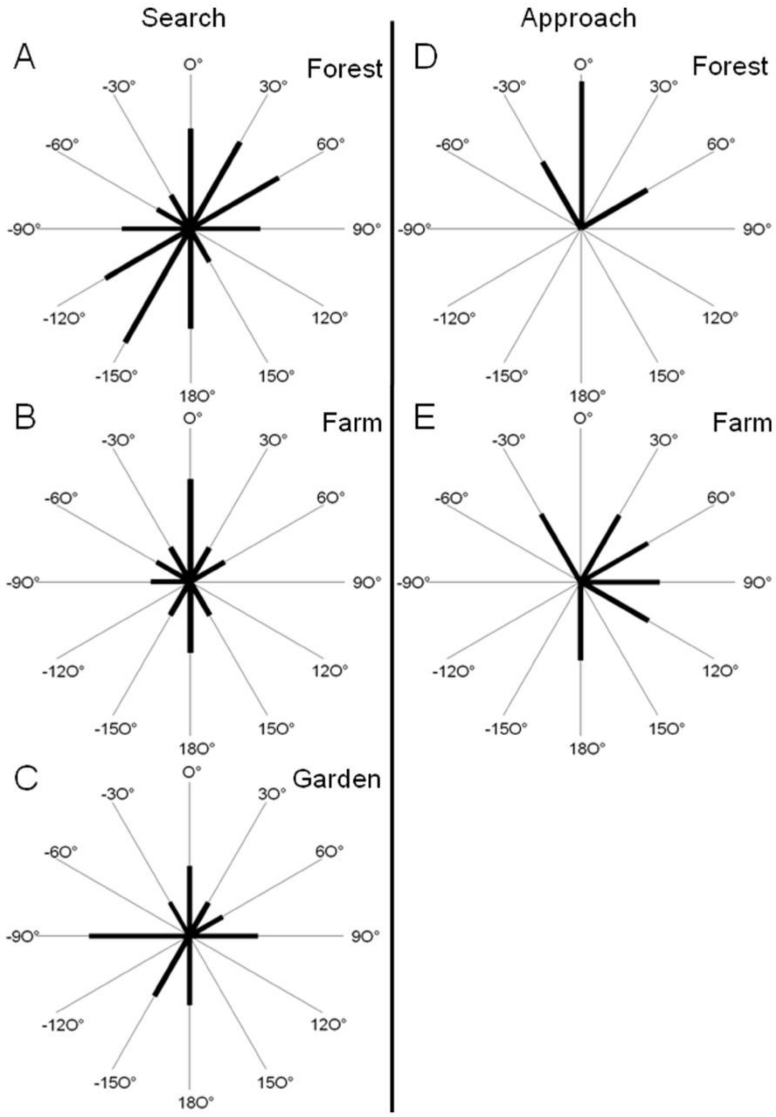

Figure 4. Angular directions of scan paths on the array. Polar histogram of angular directions of apparent and real scan paths from sequences recorded at the three edge habitat recording sites. Each of the indicated directions contains all scan path directions within a ±15° wide sector. The lengths of the black lines indicate numbers of observations. A–C depict scan path directions of search signals, and D and E represent scan path directions of approach signals. (A) forest road (180 search calls during 14 sequences), (B) farm (92 search calls during 8 sequences), (C) garden (78 search calls during 6 sequences), (D) forest road (60 approach calls during 12 sequences), (E) farm (21 approach calls during 4 sequences).
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determined by the duration of the fixation time (100–600 ms) and reaches values from ~ 2–10 updates per second [29].

Good visual search performance is essential for survival, hence many efficient strategies for selecting fixation points have evolved in mammals [30]. The scanning angles covered by head and eye

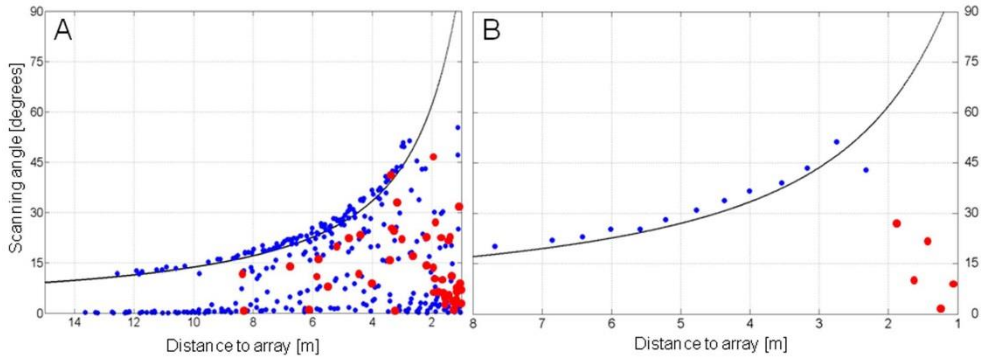

Figure 5. Apparent and real scanning angles. Apparent and real scanning angles of pipistrelle bats flying towards the microphone array. Angles are measured from the bat's position and between successive apparent or real beam maxima on the array plane. The black line indicates the calculated maximum angle in the vertical plane that can be measured with the microphone array according to its dimensions and distance from it. (A) Scanning angles of 414 calls measured during 18 flight sequences at the forest and the farm. (B) Scanning angles of a single approach sequence at the farm. Apparent (blue dots) and real (red dots) scanning angles are indicated.
doi:10.1371/journal.pone.0060752.g005

movements in the human visual field are task-dependent [21]. Depending on whether humans are looking at a scene or searching a target within that scene, their scanning behavior can greatly differ [21,31]. In bats, the sequential sampling of the environment by "acoustic gaze saccades" or scanning movements of the sonar beam also varied according to the task being performed.

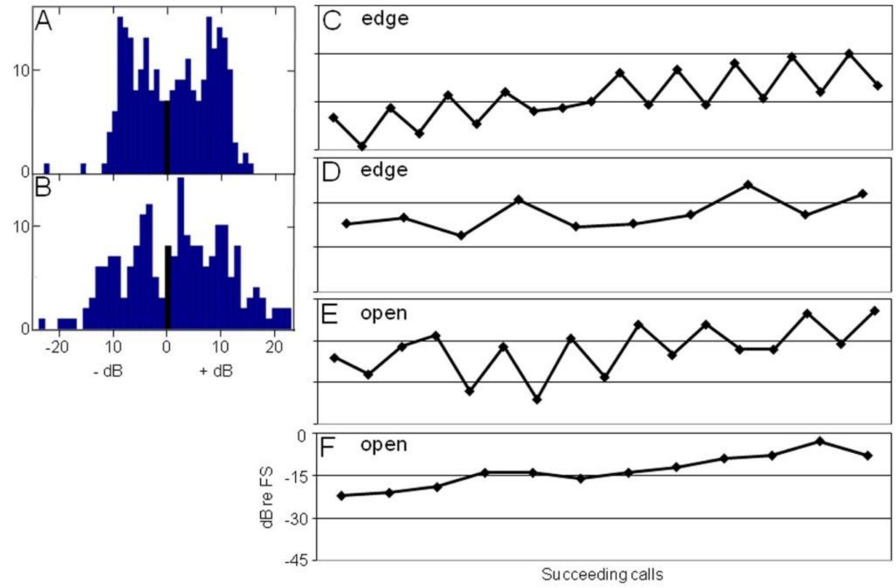

Figure 6. Comparison of scanning behavior in edge and open space. Scanning behavior of bats flying in an edge and open space derived from single microphone recordings. (A) Histogram of differences in SPL between consecutive signals in edge space (249 calls during 15 sequences) as compared to (B) histogram of differences in SPL in open space (203 calls during 20 sequences). Exemplary call sequences with distinct alternating changes of signal SPL in edge space (C) and in open space (E). Examples with more irregular changes in signal SPL for edge space (D) and for open space (F).
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Bats searching for prey in open space often made alternating acoustic gaze saccades by head movements. Sound sequences with irregular or no scanning movements were also recorded. The type, spatial distribution, and abundance of prey may determine which scanning behavior produces the highest success rate in a given situation (Figure 6).

In the three edge situations, scanning behaviors were found that shared similarities to those recorded in open space. Often, the beam was directed back and forth but also irregular scanning or no scanning was observed (Figure 6). In this situation, the bats had to perform two tasks in parallel: spatial orientation and prey detection. Site-dependent differences in scanning angle indicate that the environment had an influence on the scanning behavior of the bats, thus documenting task-specific behavior. At the farm, bats kept acoustic contact with the house wall on their left side by primarily moving their beam up and down. In the garden the bats circled while foraging, and made mainly right/left scanning movements. Before flying a curve to the right the bats occasionally aimed their sonar beam in this intended direction (Figure 4A–E). This "foresight" behavior in flight direction has already been described [11]. When flying along the forest road, bats utilized diagonal scanning movements, which may indicate the use of vegetation edges on both sides of the road for spatial orientation. The scanning movements also increased the search volume, which may improve the chance for prey detection.

Bats switch to approach behavior in three different echolocation situations: obstacle avoidance, prey pursuit, and landing. Here we could only investigate the obstacle avoidance behavior of bats approaching the microphone array. During the approach to the array, the scanning angles were reduced and the beam maxima of succeeding pulses stayed on the target of interest for a longer time, e.g., one pipistrelle bat flew through the microphone array and moved the beam with small changes of the scanning angle around the sector which was later passed. (Figure 3A, center plot). For the two other approach situations, landing and prey pursuit, previous studies suggest that bats lock their beam onto the target they are interested in [10,15]. This "locking in" on a target with the sonar beam can be compared to smooth pursuit movements in vision which keeps the target of interest on the fovea, and compensates for angular displacements produced by movements of either the target, the observer, or both [32].

We found that echolocating pipistrelle bats use a "saccade and fixate" strategy similar to vision. Since alternating signal amplitudes are also found in other bat species it is most likely that this finding can be generalized for all bats. By using these scanning movements bats are capable of finding and exploring targets in a wide search cone pointing in flight direction. We also found that the scanning behavior is task specific. However, more data collected with larger arrays are needed to fully understand how the scanning behavior is connected to specific echolocation tasks.

Materials and Methods

Ethics Statement

No specific permits were required for the described field studies since only sound recordings were made and no specimen were sampled and/or handled. No specific permits were required for the locations where recordings took place. Private land was accessed with the permit of Laurent Arthur from the Muséum d'Histoire Naturelle de Bourges, France. Field studies did not disturb endangered or protected species.

Animals and Recording Sites

Edge space recordings of the common pipistrelle bat (*P. pipistrellus*) were made at three locations in Central France (referred to as forest, farm, and garden) between June 30–July 27, 2009, and October 5–October 9, 2010, between 21:00 and 02:00 hours (MEZ). The three recording sites were chosen to represent three different echolocation scenes: bats flying along a house wall on their left side (farm; Figure 7B), bats circling in a garden in front of a barn (garden; Figure 7C), and bats flying along a straight forest road with dense vegetation on both sides (forest; Figure 7A). At each recording site and recording date several individuals with different terminal frequencies were observed, however the possibility of pseudoreplication of individuals to some degree cannot be completely excluded. At all three sites the microphone array was positioned perpendicular to the flight paths and only bats flying towards the array were recorded. On the farm, the lowest microphone row was positioned 1.6 m above a stony ground surface, the house wall on the left side was ~ 2 m away, with the house roof partially covering the array at a height of 6.5 m. A few trees on the right side were more than 10 m away at this site. In the garden, the lowest microphone row was 1.7 m above pasture-covered ground with ~ 1 m distance to the corner of a barn on the right side and 1–2 m to the bushes on the left. The bats circled in the more open area in front of the barn and the array. At this location, bats never flew past the array. In the forest, the array was positioned on a tarmac forest road forming a 10 m wide gap. The distance of the array to the forest edge at the left was ~ 2 m and ~ 4 m to the right. The lowest microphone row was 1.6 m above the road with foliage-covered ground on both sides.

Open space recordings of *P. pipistrellus* producing typical long open space signals were obtained in a grassland habitat next to a lake shore near Tübingen, Germany, on June 2, 2007, from 23:00 to 23:30 hours (MEZ).

Experimental Setup

Recordings in the three different edge space situations were made using a vertical planar microphone array with 16 microphones arranged on nylon strings (ø 0.7 mm), facing perpendicular to the array plane, and forming a 4×4 grid. The strings were attached to an aluminum frame (4×4 m). The microphones were equally spaced 0.8 m apart on both the horizontal and vertical axis (Figure 1). We used nearly omnidirectional Knowles (FG-23329, Itasca, IL, USA) microphones with known angular sensitivity at different frequencies fixed in small custom-made housings. The recorded signals were amplified using a custom-made amplifier. After starting the recording manually, the signals of each of the 16 microphones were digitized by two 8-channel National Instruments (NI-PXI 6123) cards at 500 kHz sampling rate and fed into a ring buffer using custom-made software (in 2009 SIMI-MOTION version 7.5.0.288 and in 2010 LabView, National Instruments Corporation, Austin, TX, USA). After stopping the recording, the four last seconds in the buffer were stored on a laptop as Waveform Audio (.wav) files. For the single microphone recordings in open space, we used a custom-made ultrasonic microphone (PC-Tape microphone, Animal Physiology, University of Tübingen, Germany) with a flat frequency response of ±5 dB from 20–130 kHz. The data were digitized at 16-bit and stored on a laptop using a sampling rate of 480 kHz. Array recordings in open space were not reasonable, as the chances of recording a bat in search flight approaching the array directly would be too low.

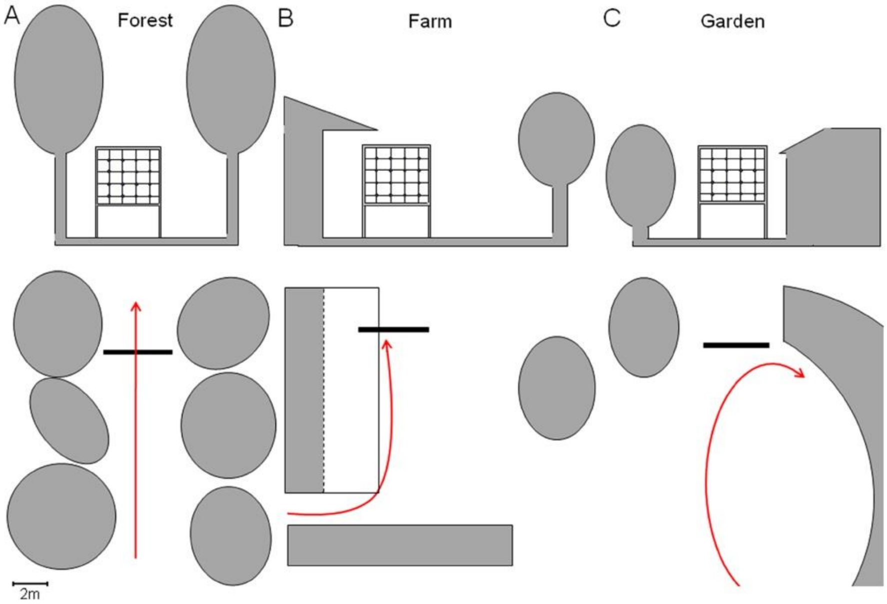

Figure 7. Recording sites in edge space. Side (upper row) and overhead views (lower row) of the three recording sites representing (A) forest road, which was lined with high deciduous trees, (B) farm, where the bats flew towards the microphone array along a house wall on their left side, (C) garden, where the bats circled in front of the array with a concave house front on their right side. Red arrows indicate typical flight paths.
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Database

From edge space recordings made using the microphone array, 32 flight sequences of pipistrelle bats containing 402 calls were analyzed. Sequences were chosen based on good signal quality (good signal-to-noise ratio at all 16 receivers) and a favorable flight path towards the microphone array. In front of the array the bats switched from search to approach behavior. We defined the beginning of the approach when two out of three sound parameters (sound pressure level, duration, pulse interval) exhibited continuous decline. 159 of the 725 calls were classified as approach calls. Only signals that were emitted at least 3 m away from the array were classified as search calls (476 signals). With this criterion we assured that no approach signals were mistaken for search signals. For the open space recordings, 22 flight sequences with 225 calls were chosen for analysis.

Flight Path Reconstruction

Flight paths in front of the array were reconstructed using a custom-made Matlab (Mathworks, Natick, MA, USA) script to calculate the position of the bat at signal emission by using the time of arrival differences (TOADs) between microphones. The TOADs between the upper left array microphone and each of the other 15 microphones were computed by cross correlating the same echolocation call. The position of the sound source was then computed using a least-squares approximation [33]. In a test with a stationary ultrasonic speaker emitting a bat-like 10 ms long FM sweep from 80–10 kHz at different positions in front of the array we found that the positioning error in all three dimensions was no more than 2–3% of the distance to the array.

Signal Analysis

The three signal parameters duration, pulse interval, and terminal frequency were measured in color spectrograms (FFT 512, Hann window, dynamic range of 90 dB) using custom-made software (Selena, University of Tübingen, Germany). Due to auto-padding and time interpolation, a resolution of $t = 0.05$ ms and $f = 215$ Hz was reached for both the array and single microphone recordings. The beginning and end of signals in spectrograms were defined using the criterion of -6 dB below best amplitude.

Calculation of Sonar Beams and Aiming

The TOAD positions along with the corresponding time stamps for each signal were used as input by Sonarbeam [34], a Matlab-based software, to calculate a polar graph of the sonar beam from the bat's perspective with color-coded SPLs (Figure 1A). Geometrical spreading loss, atmospheric attenuation, and the individual microphone angular sensitivity were each accounted for.

For each reconstructed beam, the direction of maximal intensity was computed and displayed as vector on the flight path with colors discerning between search calls (blue) and approach calls (red) (Figure 1B–E). The reconstructed beam maxima are referred to as real, when beam maximum values fell within the array, or as apparent, when the maximum values fell either at the border or outside of the array. Preliminary tests with an artificial sound source indicated that the accuracy of beam reconstruction was sufficient to determine changes in angular orientation of the sonar beam. Position errors for the beam maximum of up to 15° were measured [35]. Real and/or apparent beam maxima of succeeding pulses were used to calculate the scanning angles. The scan path which connects successive beam maxima indicates scanning movements (Figure 3). The angular direction of scan path sections between calls was determined and displayed in six 30° bins (Figure 4).

SL Determination

For the calculations of search phase source levels (SL), only calls of bats flying towards the array and emitted between 3–10 m were used. The frequency range was limited to 40–60 kHz and sound pressure levels (SPL) are given in dB SPL *re* 20 µPa at 1 m (rms). Only 141 of the recorded search calls were centered within the array plane in such a way as to ensure that only real beam maxima, and not apparent beam maxima, were measured. Statistically, the apparent source levels (ASLs) did not differ from the SLs (e.g. for the forest site: $F_{1,196} = 0.44$, $p > 0.5$), therefore both ASLs and SLs were included in analyses.

Statistics

Statistical analysis was performed in JMP (SAS Institute Inc., Cary, NC, USA). To test for differences of signal parameters at the three recording sites, a one-factorial ANOVA was performed followed by a post-hoc Tukey-Kramer test using standard significance criteria ($p \leq 0.05$). To avoid pseudoreplication when calculating the mean signal parameters from sequences containing a different number of signals, only the mean of each sequence was determined and used for further analyses.

Acknowledgments

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Author Contributions

Wrote the TOAD script used in analysis: JCK. Conceived and designed the experiments: AS AD HUS. Performed the experiments: AS. Analyzed the data: AS HUS. Contributed reagents/materials/analysis tools: AD HUS. Wrote the paper: AS AD HUS.

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5.3 Bifunctional echolocation in the bat *Barbastella barbastellus*: Two different signals of equally low source level are emitted through mouth and nose in different directions

This manuscript is part of Anna-Maria Seibert's doctorate thesis. I assisted in array design and in the development of the recording software. I adapted the employed localization software, provided additional matlab programs, discussed the data and results and commented on the manuscript.

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Figure 17: The 6 m tall chain array design set up in France, Photo: Anna-Maria Seibert.

Abstract

Barbastelle bats (*Barbastella barbastellus*) prey almost exclusively on tympanate moths. While foraging, this species alternates between two different signal types. We investigated whether these signals differ in emission direction or source level (SL) as assumed from earlier single microphone recordings. We used two different settings of a 16-microphone array to determine SL and sonar beam direction at various locations in the field. Both types of search signals had low SLs of 81 and 82 dB SPL as compared to other aerial-hawking bats. The two signal types were emitted in different directions. Type 1 signals were pointed downward and type 2 signals were pointed upward. The angle between beam directions was approximately 70°. Barbastelle bats are able to emit signals through the mouth and the nostrils. As the mouth and nostril openings are roughly perpendicular to each other, we conclude that type 1 signals are emitted through the mouth while type 2 signals and approach signals are emitted through the nose. We hypothesize that the “stealth” echolocation system of *B. barbastellus* is bifunctional. The more upward directed nose signals are used for search and localization of prey. Their low SL prevents an early detection by eared moths but come at the expense of a strongly reduced detection range for the environment below the bat. The more downward directed mouth signals have evolved to compensate for this disadvantage and are used for spatial orientation. This bifunctional echolocation system is adapted to the selective foraging of eared moths and is an excellent example of a sophisticated sensory arms race between predator and prey.

Keywords: bat, echolocation, bifunctional, source level, microphone array

Introduction

Barbastelle bats (*Barbastella barbastellus*) are one of the most specialized Palaearctic bats [1] preying almost exclusively on lepidoptera, especially on small tympanate moths (up to 99% by volume; [1-4]. Barbastelles belong to the guild of edge space aerial-hawking foragers [5,6] and forage mainly above the canopy [1] but also in woodlands, open grasslands, and rocky landscapes [7,8].

The echolocation behavior of *B. barbastellus* is unique among the European Vespertilionids. Barbastelle bats emit two different search call types [1,3,9-13], designated as type 1 and type 2 calls [14]. The more stereotyped type 1 FM-signals are shorter and sweep from 36 – 28 kHz, while type 2 signals are longer and cover a frequency range of 45 – 32 kHz. Research using single microphone recordings suggested that the two call types of *B. barbastellus* varied in amplitude or in emission direction, with type 1 calls having higher amplitudes than type 2 calls [1,9,13,14]. Whether this variation in amplitude is caused by a change in emission direction by vertical head movements [14], or a deliberate change of source level (SL), could not be determined. Additionally, different emitters could be involved with the two signal types as bats have been observed taking off and emitting echolocation signals with their mouth shut [15]. This same observation was made earlier [16]. When either the nose or mouth was experimentally plugged, *B. barbastellus* could still fly and orientate and emit signals with similar oscillograms. Only when both the mouth and nostrils were plugged did the bats fail to orientate during flight [16].

Several hypotheses have been suggested to explain the function of these two alternating signals. The more narrowband low-frequency type 1 signal could be better suited for detection, whereas the higher and more broadband type 2 signal could be better suited for the exact localization of targets [13,14]. Recent research has found evidence that barbastelle bats use signals that are 10 – 100 times weaker than those of other aerial hawking bats to prevent an early detection and evasive behavior by moths. While moth neurons always react to loud search signals emitted by *Nyctalus leisleri* before the bat was able to hear the moth echoes, these same neurons failed to detect the “stealth echolocation” signals of *B. barbastellus* [17].

Here we address whether the alternating signal amplitude previously recorded using single microphones reflects a change in emission direction, or alternatively a change in signal sound pressure level. To accomplish this, we used a large microphone array to record the echolocation signals of barbastelle bats while flying towards the array and emitting both types of search signals. With this method it was possible, for the first time, to determine both the SL and the emission direction of the recorded signals. We also investigated how nose and mouth morphology contributes to emission direction in barbastelle bats.

Results

Flight and echolocation behavior

We recorded barbastelle bats flying towards the microphone array after leaving their roost. The bats emitted type 1 and type 2 signals in an alternating fashion. Type 1 signals always had a higher sound pressure level (SPL) at the lower microphone whereas type 2 signals had a higher SPL at the upper microphone. At a distance of 1-2 m to the array the bats switched to approach calls. During this approach phase they no longer emitted type 1 calls, and transformed type 2 calls into broadband approach calls (Figure 1A,B). Bats had an average flight speed of 5.3 m/s and flew 2-4 m above ground.

Echolocation call parameters

All measured parameters differed significantly between the two search signal types, except for SL (t=1.90, p>0.0642). The mean pulse interval between a type 1 call and a following type 2 call was 67.9 ± 12.8 ms, and between a type 2 call and its following type 1 call 59.3 ± 11.4 ms. The pulse interval between call type 1 and the consecutive call type 1 averaged 144.0 ± 60.0 ms. The pulse interval between two type 2 calls was 99.8 ± 31.8 ms. Mean call duration of type 1 calls was 1.7 ± 0.4 ms, type 2 calls had a mean duration of 2.5 ± 0.4 ms (t=7.10, p<0.0001). Start frequency of type 1 was 35.9 ± 1.3 kHz, that of type 2 44.3 ± 0.9 kHz (t=25.89, p<0.0001). Type 1 signals ended with a terminal frequency of 31.2 ± 0.8 kHz, type 2 calls ended at 35.1 ± 1.9 kHz (t=9.19, p<0.0001). Bandwidth in type 1 calls was 4.7 ± 1.0 kHz, that of type 2 calls was 9.2 ± 1.9 kHz (t=10.37, p<0.0001). The peak frequency of type 1 calls measured 33.6 ± 1.1 kHz, and for type 2 calls 40.1 ± 1.2 kHz (t=18.99, p<0.0001). The apparent SL of type 1 signals averaged 79.9 ± 5.1 dB SPL and that of type 2 signals was 82.4 ± 3.6 dB SPL. Type 1 calls had an absolute SL of 80.9 ± 7.5 dB SPL and type 2 calls had a SL of 82.0 ± 4.1 dB SPL (Table 1).

Sonar beams and beam direction

The reconstructed sonar beams showed a clear pattern of alternating directions. Type 1 signals usually had its apparent beam maximum on the lower part of the array, while type 2 calls showed apparent beam maxima on the upper array edges (Figure 2J,K). This pattern was also found in the recordings of the 6 m high polar array (Figure 2M,N). The fact that approach signals also pointed upward suggests that these signals are derived from type 2 signals. During approach, bats focused on the array resulting in beam maxima within the array (Figure 2L,O). A complete sequence of alternating signals and three approach signals is depicted in Figure 2A-H. Vectors pointing from the bat to the apparent or real beam maximum indicate beam direction (Figure 3). These reconstructions, too, showed the distinct alternating pattern of type 1 signals pointing downward and type 2 signals pointing upward.

Apparent angles between call types

With the square array we measured an apparent mean vertical angle based on the apparent direction of 66 type 1 beams at -13.9° ± 16.9° relative to flight direction, whereas the mean vertical angle based on the apparent direction of 88 type 2 calls is 20.0° ± 20.2° relative to flight direction (Figure 4A). This resulted in an apparent mean vertical angular offset between the two search signals types of at least 33.9°. The geometric limits of the microphone array did not allow measuring larger angles. It is likely that real angles between the flight path and the calculated apparent beam vector exceed these reported apparent angles. The small offset of the mean to the right side is probably due to the angle in which the bats approached the array after leaving their roost. The pulse-to-pulse path of beam maxima on the array plane of one sequence is illustrated in Figure 4B. Type 1 calls clustered on the lower right edge of the array while type 2 calls were found towards the upper left edge. Approach calls stayed within the array limits and indicate that the bat was focusing on the obstacle during approach.

The 6 m high chain array was used to sample a larger vertical section. Again many of the apparent beam directions either point on the lower or the upper array edge, particularly when the bat was at distance. Again, type 1 signals pointed downward and type 2 signals upward. Once bats closed in on the array, some calls were within the measurement limits (Figure 5). With the chain array, we found apparent vertical angles between the two call types of up to 88° (Figure 6). Starting at 3 m distance from the array, where array height did not limit the measured angles, the vertical angles between signal types ranged between 50-70°.

Echolocation behavior based on head anatomy

It is assumed that barbastelle bats are able to emit echolocation calls through both their open mouth and nostrils [15,16]. In barbastelles, the mouth and nose openings point in different directions, with the nostril openings roughly perpendicular to the mouth opening. The nostrils are tilted upwards and open into a system of embedded lacunas leading all the way from the nose to the beginning of the ears, beyond and around the tragus. Comparing the external anatomy of the snout of several Vespertilionid species it is conspicuous that the embedded lacunas and upward pointing nostrils described for *B. barbastellus* are also found in the genus *Plecotus*, but not in other genera of this family whose nostrils open in a more forward direction (Figure 7). Lump-nosed bats (*Plecotus spec.*) are known to emit sounds through their nostrils as well [18]. Together with *Plecotus* species barbastelle bats belong to the tribe of Plecotini, and are therefore closer related to each other than to other species within the Vespertilionids

Discussion

Here we present new results on the SL of type 1 and type 2 signals and on the change of beam direction in *B. barbastellus* during search and approach flight. The sound recordings with just two microphones, one from the top row and one from the lowest row of the 16-microphone square array, indicated that the beam direction changes between call types. Type 1 signals always had a higher SPL at the lower microphone whereas type 2 signals had a higher SPL at the upper microphone (Figure 1). This change in beam direction was confirmed by the alternating sonar footprints on the array; type 1 signals had their beam maxima on the lower part and type 2 signals had theirs on the upper part of the array plane (Figure 2).

The determination of apparent and real beam directions allowed more detailed evaluation of signal SLs and of the changes in beam direction. In the cases when the beam maximum was within the array the real SL was approximately the same for both call types with ca. 81 dB for type 1 and 82 dB for type 2 calls. The apparent SL of signals which were not centered on the array did not greatly deviate from these values (Table 1). This result strongly suggests that the SPL variations between call types found in single microphone recordings are the result of changes in beam direction [14] and not of changes in emission SPL. Beam direction clearly switched between up and down as the bats alternated their call types (Figure 3). At the square array as well as at the chain array nearly all type 1 signals had an apparent beam maximum at the lower end of the array whereas type 2 signals had the apparent maximum at the upper end. When measuring the apparent call angles between flight and beam direction we found that the directions of most type 1 calls were below flight direction whereas those of most type 2 calls were above.

The measured vertical angles between the alternating beam emission directions were estimated as smaller than the real angles since most beams produced only apparent maxima - even at the 6 m chain array. Only at distances below 3 m, the measured angles were not limited by the borders of the array (Figure 5 & 6). There we found apparent angles that cover a range of 50° – 70°. Under the assumption that the largest apparent angles are close to the real angles, we conclude that the real angular separation between the two beam directions was approximately 70° (Figure 6).

In summary, we found that the two types of search signals of *B. barbastellus* have about the same SL but are emitted in different directions in the vertical. The beams are separated by about 70° and the beam directions of type 1 signals are below flight direction whereas those of type 2 signals are above.

Beam direction of approach calls

The apparent and real sonar beam directions of approach calls resemble those of type 2 calls and mostly point in the upper area of the array (Figure 2-5). Even the recordings with only one lower and one higher microphone showed that the higher microphone recorded not only type 2 signals but also approach signals with higher amplitude (Figure 1). The similarity in beam direction supports the conclusion that the broadband approach calls are derived from type 2 signals, as evidenced by sonagrams which show how type 2 calls morph into approach calls (Figure 1), [14,23].

A hypothesis to explain the alternation between two emission directions

From single microphone recordings it was concluded that the amplitude variation between type 1 and type 2 signals indicate changes in beam direction in the vertical and might be caused by head movements in an up and down direction [14]. Another explanation for the observed change in emission direction could be that the upward directed type 2 signals are emitted through the nose and downward directed type 1 signals through the mouth. This hypothesis is supported by the observation that *B. barbastellus*, when taking off, emitted type 1 signals through the mouth and type 2 signals through the nose [15]. The possibility of switching between mouth and nose emission is supported by the finding that even if either the mouth or the nose of bats are plugged they are able to fly, to avoid obstacles, and to emit echolocation signals [16]. It was found that *B. barbastellus* and *Plecotus auritus* are able to emit signals either through the nose or through the mouth and that the oscillograms of the mouth and nose signals look rather similar. It was also reported that *P. auritus* flies with mouth shut and that the echolocation calls are emitted through the nostrils which open upwards [18].

The switching between mouth and nose emission would explain the reported differences in SPL between call types in single microphone recordings only if the beams of the mouth and of the nose signals point in different directions. When looking at the head anatomy of *B. barbastellus* we found that the opening direction of the nostrils is nearly perpendicular to the direction of the mouth opening (Figure 7). If we assume that the pointing direction of nose and mouth opening determines the beam directions of the emitted signals we predict that the nose beam should be almost perpendicular to that of the mouth. This prediction corresponds well with our measurements that indicate a separation angle of about 70° in the vertical. We therefore conclude that *B. barbastellus* vary their emission direction in the vertical of about 70° by emitting type 1 signals through the mouth and type 2 through the nose. The degree of angular separation between nose and mouth beam is determined by the anatomical relation between nostrils and mouth. This anatomical relation is fixed such that the angle between the two emission beams is likely invariable.

The emission directions of this fixed system are additionally dependent on the aiming of the head. We found that the beam directions of most type 1 calls were below flight direction whereas those of most type 2 calls were above. This implies that in horizontal flight the mouth opening points downward and the nostrils point upward. *Pipistrellus pipistrellus*, a species that emits signals only through the mouth, is able to move its head and with it the beam direction [19]. We therefore assume that *B. barbastellus* also has the ability to move its head around a roll, pitch and yaw axis and with it the beams of the anatomically fixed nose and mouth emitters. Some of our data indicate that the bats also make head movements. The beam movement between succeeding signals was not only in the vertical but also tilted to the side around a roll axis which is indicated by the measured horizontal angles in Figure 4A. The scan path of an example flight in Figure 4B also shows that the bat was capable of head turning so that the beam moved from right/down to left/up and back. Another hint of head movements around the pitch axis comes from directional changes of the approach signals in Figure 4 & 5. The direction of these nose signals moves from the upper end of the array more to the centre indicating that the bats fixate a target with the nose beam. We predict that such a fixation with the nose beam will also occur in bats approaching an insect.

Adaptive value of emitting two types of search calls with similar SL in different directions

B. barbastellus forages near vegetation during aerial-hawking and preys mainly on tympanate moths [3,11,17]. According to this foraging behavior, barbastelles are attributed to the guild of “edge space aerial-hawking foragers” that search for prey near background targets [6]. Foraging and echolocation behavior of bats have been adapted to the task they have to perform while searching and acquiring food. All edge space foragers share similar adaptations, and their echolocation systems display many similarities. However, this does not exclude species-specific differences that reflect niche partitioning within guilds [6]. If we compare the echolocation behavior of *B. barbastellus* with that of other edge space foragers we find many similarities but also some distinct differences which may account for niche differentiation.

The signal pattern of foraging *B. barbastellus* generated only by type 2 search signals and approach signals is rather similar to that of other edge space aerial-hawking foragers [14,20]. The bats emit (likely in the rhythm of the wing-beat indicated by the intervals between type 2 pulses of 120 – 130 ms) shallowly downward modulated search signals of moderate bandwidth (frequency range of 45-32 kHz). The signals are varied in duration (around 8-3 ms), most likely in relation to the distance to the background. The initial more shallow part of the signal improves detection and the steeper modulated terminal part improves localization of prey. Sometimes the bats skip a sound emission which is indicated by a long interval between the type 2 signals. After detection of prey with a long type 2 signal the bats switch to broadband approach signals (frequency range of 52 – 23 kHz). The first signal of an approach may be even longer than the preceding type 2 signal (transition signal according to [20]) but afterwards signal duration and pulse interval are reduced in the typical way as in other aerial-hawking species approaching prey. The approach ends with a typical buzz which is indicated by pulse intervals below 8 ms. Rather often the buzz is quite long and often interrupted by short intervals. The prolonged buzz most likely indicates that the bats pursue prey which tries to escape. In summary, in foraging *B. barbastellus* the pattern of only type 2 and approach signals is rather similar to that of other edge space aerial-hawking foragers.

However, we also found distinct differences when comparing the echolocation behavior of *B. barbastellus* with that of other edge space aerial-hawking foragers. One big difference is that the type 2 and the approach signals are most likely emitted through the nose. The emission of signals through the nose is also found in bats of the genus Plecotus which - together with the barbastelles - belong to the tribe Plecotini. This phylogenetic relationship probably indicates that the two genera had a common ancestor with nose emission [21]. It is striking that the nostril alignment of those two genera differs from the other Vespertilionids in that it contains more cavities and the nose openings are turned upwards (see also Figure 7).

But why are other genera within the family of Vespertilionidae able to forage with mouth-bound signals alone while barbastelle bats have evolved two different emitters and use two different signal types with equal SLs (81 – 82 dB) far below those of other aerial-hawking foragers (111 - 101 dB, [22])? This rather low SL has been interpreted as an adaptation that allows *B. barbastellus* to hunt successfully for tympanate moths. Many moths can hear and react with escape responses as soon as they detect an approaching bat [2,23,24]. Most edge space aerial-hawking foragers have high SLs which provide long detection distances. However, these high SLs have the disadvantage that moths can detect the bats early enough to initiate an often successful escape maneuver. Goerlitz et al. (2010) [17] were the first to propose that *B. barbastellus* might use some kind of "stealth echolocation" and produces search signals with low SL that are inconspicuous to eared moths. They determined a SL for type 1 signals of only 71 dB re 1 m (rms). We assume that this value is too low as their method probably delivered weaker apparent beam maxima instead of louder real beam maxima. However, the histograms of their SLs (Figure 3B in [17]) have an upper limit near 81 dB re 1 m which corresponds very well to the average SL of type 1 signals of our measurements.

For *B. barbastellus* foraging for large moths with a target strength (TS) of -36 dB (re 1 m) the detection distance is about 3.0 m if we assume a best frequency of 40 kHz, a detection threshold of 20 dB, a temperature of 15 ° Celsius, and a humidity of 50%. Under similar condition *N. leisleri* could detect this prey over a distance of 8.5 m if we assume a SL of 107 dB and a best frequency of 28 kHz (see [25]). The neural maximum detection distances of the moth *Noctua pronuba* for echolocation signals of foraging *B. barbastellus* and *N. leisleri* was measured in the field [17]. The more sensitive A1 neuron of the moth always reacted to the search signals of *N. leisleri* before the bat heard the moth echoes. The moth's A1 detection distance was with 33.2 m far beyond the 8.5 m detection distance of the bat calculated from our data. This early warning gives eared moths the possibility to start with escape movements in time. In foraging *B. barbastellus* the moth's A1 neuron detection distance of 3.5 m was close to the bat's detection distance of 3.0 m which we calculated from our data. This suggests that eared moths have far less chances to escape barbestelles by evasive movements. Therefore we agree with Goerlitz et al. (2010) [17] who stated "*B. barbastellus* uses a stealth echolocation strategy by emitting low-amplitude calls, a strategy previously suggested by Fenton & Fullard (1979) [26] and by Surlykke (1988) [27] and now supported with field-based measurements".

In contrast to Goerlitz et al. (2010) [17] we do not assume that *B. barbastellus* use type 1 signals as search signals for prey. We hypothesize that these signals have a different function. The SLs of the type 1 and type 2 signals of *B. barbastellus* are 20 – 25 dB lower than the SLs of other edge space aerial-hawking foragers. This has the advantage that *B. barbastellus* can come very close to eared moths without provoking evasive movements, but at the cost of a highly reduced detection distance for prey and background targets. The comparison of type 2 signals with search signals of *N. leisleri* showed a reduction of the detection distance for prey to about one third which would reduce the search volume to about one-twenty-seventh. Additionally, the detection distance for vegetation ahead is strongly reduced from 23 m for a forest edge in *N. leisleri* to 7.9 m in type 2 signals of *B. Barbastellus* (all calculations according to [25]). In *B. barbastellus* foraging above a forest canopy with the beam pointing slightly upward, the detection and the evaluation of echoes from the canopy below becomes even more difficult. The directionality of signal emission and also of echo reception substantially reduces the SPL of type 2 echoes from below. A reasonable reduction of the echo SPL by at least 20 – 30 dB decreases the detection distance to the forest below to only 2.1 – 0.6 m. This short detection distance of the forest below would make it difficult for the bat to forage successfully in this biotope. However, a signal like the type 1 signal that is emitted downward improves the chance to detect and evaluate forest echoes from below. A type 1 signal that is directed downward allows detection distances of up to 8.6 m for a forest canopy, 13.6 m for a meadow, and 18.1 m for a water surface. We therefore conclude that the downward directed type 1 signals have been evolved to control the position and the nature of the background below the bat. Such a strategy has previously been suggested by Denzinger et al. (2001) [14]. With downward directed type 1 signals *B. barbastellus* overcomes the disadvantage of the stealth strategy with the rather short detection distances for the targets below the bat due to the low emission SLs of the more upward directed type 2 signals. This argument is supported by the result that type 1 signals are always rather stereotyped and are not changed in relation to the foraging situation [14].

A different argument to explain why *B. barbastellus* evolved two types of echolocation signals was used by Barataud (2004) [20]. He suggested that bats use two signal types which differ in intensity, structure, and frequency to deceive tympanate moths by mimicking the presence of two bats at different distances with sufficiently low repetition rates so as to not provoke the prey's escape behavior. Goerlitz et al. (2010) [17] and the present study suggest that *B. barbastellus* uses the stealth strategy to improve their hunting success. This, however, makes it unlikely that the two signal types have evolved to deceive the moths, as both signal types have low SLs and cannot be heard by the moths.

We found that foraging *B. barbastellus* emit two signal types of equally low SL in different directions. In relation to flight direction the beams of type 2 signals point upward, and that of type 1 signals downward. The beams are separated by a fixed vertical angle of approximately 70°. Barbastelle bats are able to emit signals through the mouth or the nose and these of mouth and nostril openings occur at a roughly perpendicular angle to each other. This suggests that type 1 signals are most likely emitted through the mouth and type 2 through the nose. In addition, this fixed double emission system can be actively adjusted up or down around the pitch axis, and tilted around the roll axis of the head. We hypothesize that the "stealth" echolocation system of *B. barbastellus* with two different signal types of low SL which are emitted in different emission directions, is bifunctional. The upward directed nose signals are used for the search of, and the approach to, prey. Their low SL prevents an early detection of bats by eared moths at the expense of a significantly reduced detection range for the environment below the bat. The more downward directed mouth signals have evolved to compensate for this disadvantage. These signals are needed for spatial orientation and biotope recognition.

Materials and Methods

Ethics Statement

No specific permits were required for the described field studies since only sound recordings were made and no specimen were sampled and/or handled. No specific permits were required for the locations where recordings took place. Private land was accessed with the permit of Laurent Arthur from the Muséum d'Histoire Naturelle de Bourges, France. Field studies did not disturb endangered or protected species.

Animals and recording sites

Barbastelle bats (*Barbastella barbastellus*) were recorded at three locations with known roosts (referred to as location # 1-3) in Central France near Bourges from June 29 – July 20 2009 between 22:00 and 23:00 hours (MEZ). The bats usually left their roost at about 22:00 hours. Before we set up the recording equipment we determined the common flight route of bats emerging from the roost. The arrays were positioned perpendicular to their main flight corridor with a distance of 6-35 m from the roost to ensure that the bats flew more or less centered on and straight towards the array. Bats approached the array at heights of 3-6 m.

Pseudoreplication by recording an individual several times cannot be completely excluded since recordings were made on consecutive nights. However, bats were leaving their roost and we assume that per night any given animal was recorded only once with approximately the same number of alternating call types.

Experimental setup

We used nearly omnidirectional Knowles (FG-23329, Itasca, IL, USA) microphones with known angular sensitivity at different frequencies fixed in small custom-made housings. Recordings were made using two different array-configurations. Most recordings were made with a **square microphone array** with 16 microphones arranged on nylon strings (ø 0.7 mm) in a 4x4 vertical planar grid attached to an aluminum frame (4x4 m). The microphones were equally spaced with 0.80 m apart on the horizontal and vertical axis facing the bats (Figure 1B). With the square array the lowest microphone row was elevated approx. 1.5-2.0 m above ground.

At one location we additionally used a setup with three 6 m long microphone chains which were kept in a vertical position by two flag poles. At the **chain array** the microphones were attached to nylon strings in a 4-8-4 grid with a vertical spacing of

2.0, 0.85, and 2.0 m respectively. The nylon strings were spaced with 0.80 m on the horizontal axis (Figure 3B,D). In that setting, the lowest microphone row was approx. 0.3 m above ground.

The recorded signals of each of the 16 microphones were amplified using a custom-made amplifier and digitized by two 8-channel National Instruments (NI-PXI 6123) cards at 500 kHz and 16 bit sampling rate and fed into a ring buffer using custom-made software (SIMI-MOTION version 7.5.0.288). After stopping the recording, the four last seconds in the buffer were stored on a laptop computer as Waveform Audio (.wav) files.

Database

24 sequences of approaching barbastelle bats recorded with the square array were analyzed; these contained 337 calls in total. One sequence originates from location #1, three sequences from location #2, and the remaining 20 sequences were recorded at location #3. These calls were all emitted at distances of 1-10 m from the microphone array. We recorded 86 type 1, 110 type 2, and 141 approach calls. With the chain array, six sequences containing 26 type 1 calls, 30 type 2 calls, and 28 approach calls from location #3 were analyzed. Sequences were chosen based on good signal quality (good signal-to-noise ratio at all 16 receivers) and favorable flight path towards the microphone array.

Flight path reconstruction

Each bat’s flight path was reconstructed using a custom-made Matlab (Mathworks, Natick, MA, USA) script to calculate the position of the bat at signal emission by using the time of arrival differences (TOADs) between microphones. The TOADs between the upper left array microphone and each of the other 15 microphones were computed by cross correlating the same echolocation call. The position of the sound source was then computed using least-squares approximation [28]. In a test with a stationary ultrasonic speaker emitting a bat-like 10 ms long FM sweep from 80-10 kHz at different positions in front of the array we found that the positioning error in all three dimensions was no more than 2-3% of the distance to the array.

Signal analysis

Several signal parameters, including call duration, pulse intervals, bandwidth, peak frequency, and start and terminal frequency were measured in color spectrograms (FFT 512, Hann window, dynamic range of 90 dB) using custom-made software (Selena, University of Tübingen, Germany). Due to auto-padding and time interpolation, a resolution of t = 0.05 ms and f = 215 Hz was reached. The beginning and end of signals were measured in the spectrograms using the criterion of -6 dB below highest amplitude. To avoid pseudoreplication in the calculation of mean signal parameters only the mean of each sequence was determined and used for subsequent analyses (Table 1).

Sonar footprint and sonar beam

The TOAD positions along with the corresponding time stamps for each signal were used as input by a Matlab-based software (Sonarbeam [29]), to calculate sonar footprints on the array plane and polar graphs of the sonar beams from the bat’s perspective with color-coded SPLs (Figure 3). Geometrical spreading loss, atmospheric attenuation, and the individual microphone angular sensitivity were accounted for. The reconstructed beam maxima (maximum value in the polar graph of a beam) are referred to as real maxima, when beam maximum values fell within the array, or as apparent maxima, when the maximum values were positioned at the border of the array. Preliminary tests with an artificial sound source indicated that the accuracy of beam reconstruction was sufficient to determine changes in angular orientation of the sonar beam. Position errors for the beam maximum of approximately 2° were measured [30].

Determining calling direction

For each reconstructed beam recorded with the square array, the direction of the real or apparent beam maximum was determined and displayed as vector on the flight path with vector colors discerning between call types (type 1 calls in red, type 2 calls in blue, approach calls in black; Figure 2). The real or apparent beam direction in relation to the flight path was also described by the azimuth and elevation angle of the real or apparent beam direction in relation to flight direction (Figure 4A). Additionally, changes in beam direction were indicated by the path of the real and apparent beam maxima of succeeding pulses on the microphone grid (Figure 4B).

To describe changes of beam direction in recordings with the 6 m high chain array we determined the horizontal and vertical angle between the center of the array and the reconstructed real or apparent beam maximum for succeeding pulses. However, the spatial resolution in the horizontal was too small for a precise beam reconstruction. Therefore we present only data describing the vertical deviation from the center of the array (Figure 5). In most recordings with the chain array, the bat passed the array on either side. The array therefore only recorded vertical cross sections of the beams and thus, our results describe only changes of apparent beam directions along the vertical.

SL determination

For the calculations of the SLs of both call types, the frequency range was limited to 20-60 kHz and sound pressure levels (SPL) are given in dB re 20 µPa at 1 m (rms). Only 14 of the recorded type 1 calls and 10 of the type 2 calls were centered within the array plane in such a way as to ensure that only real beam maxima, and not apparent beam maxima, were measured. However, the apparent SLs and the absolute SLs differed only slightly, therefore both ASLs and SLs were included in analyses (Table 1).

Methodological limits

We investigated the SL and the variation of beam direction with arrays of 16 microphones. This approach had the advantage that a substantial part of the acoustic beam could be sampled. The sampled part of the beam increased as the bat closed in on the array. When the maximal intensity of the beam was within the array, we could determine the SL of the signals and the exact beam emission direction. When the beam maximum was not within the array, the apparent beam maximum and the apparent beam direction still delivered information on the SL and on beam movements between succeeding signals. We are aware that the array design also posed certain limits. The square array (4x4 microphones) was too small in the vertical to record centered beam maxima in a sequence of succeeding type 1 and type 2 signals. Either the bats were too far away, and thus the beams pointed above and below the array, or they were close enough but then bats switched to approach calls. The chain array (4+8+4 microphones) had sufficient vertical but poor horizontal spatial resolution. However, apparent beam maxima of succeeding pulses gave some indications about the directional changes of the beam. They also indicated whether the beam direction was above or below flight direction (Figure 4A).

Statistics

Statistical analysis was performed in JMP (SAS Institute Inc., Cary, NC, USA). To test for the differences between the two signal types a student's t-test was performed followed by a post-hoc Tukey-Kramer test using standard significance criteria ($p \leq 0.05$). To avoid pseudoreplication when calculating the mean signal parameters from sequences containing a different number of signals, only the mean of each sequence was determined and used for further analyses.

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Figures

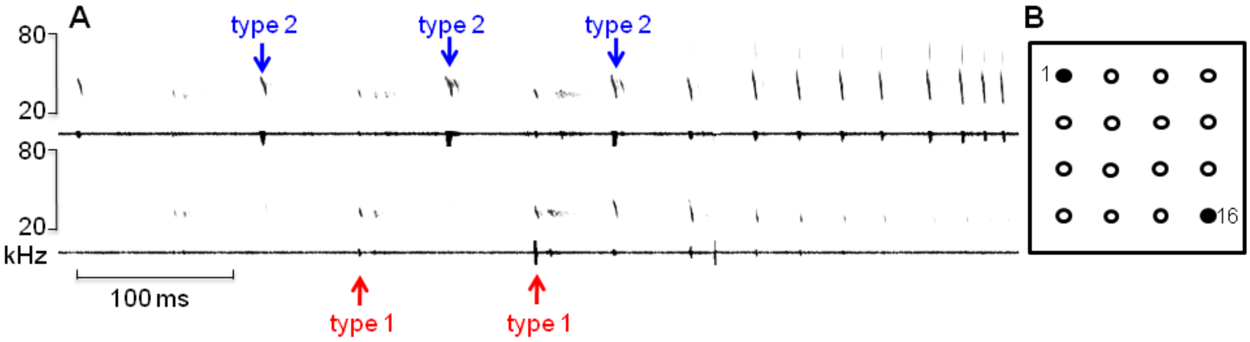

Figure 1: Echolocation signals of *B. barbastellus* recorded from different microphone positions. (A) Spectrograms and oscillograms of echolocation signals emitted when flying towards the array (FFT: 256, Hann window). (B) The signals in the upper half of the graph were recorded by the top left microphone of the array (number 1), the signals in the lower half of the graph by the lowest right microphone (number 16). Type 2 and approach signals were recorded at higher amplitudes of the upper microphone, whereas type 1 signals have higher amplitudes in the recordings of the lower microphone.

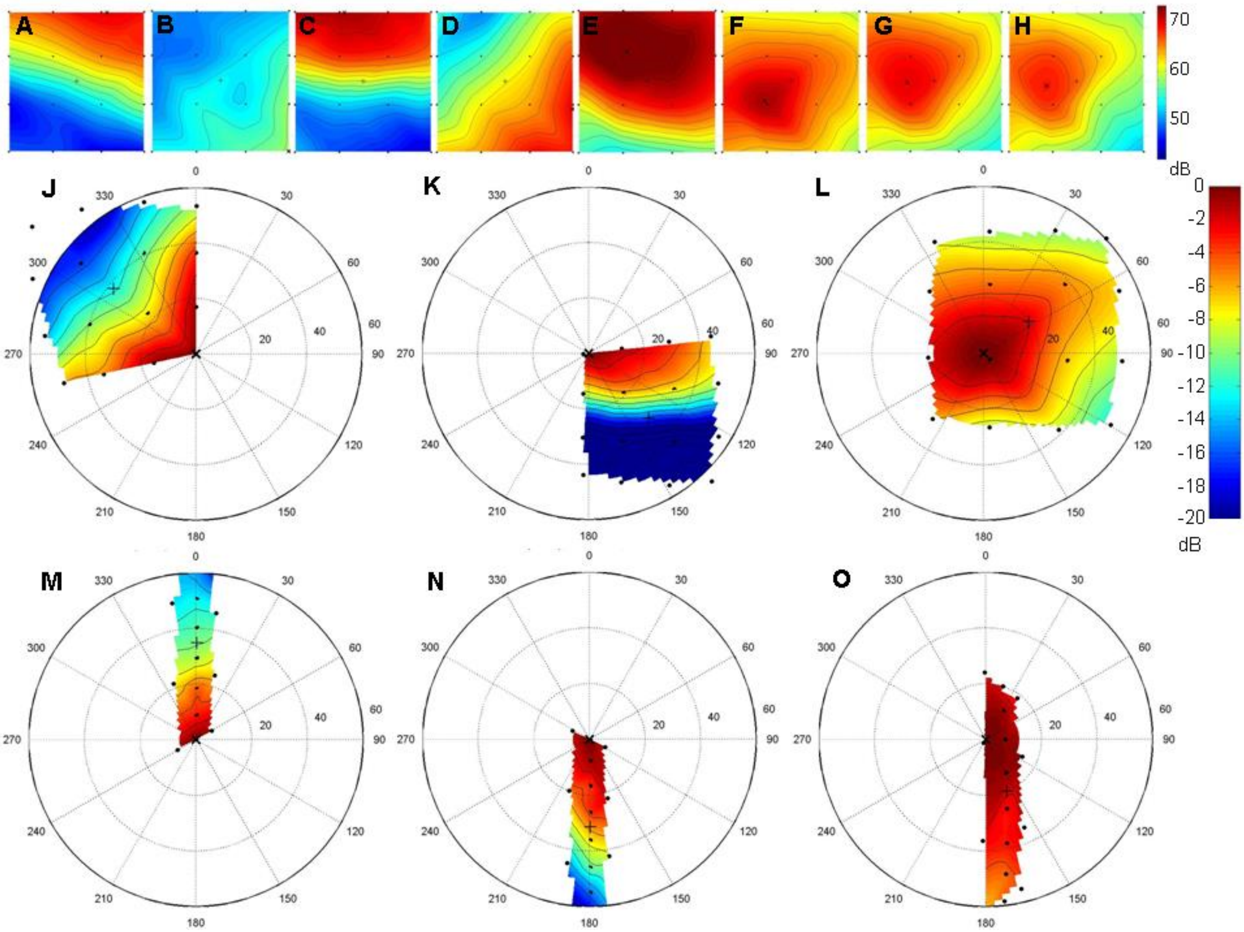

Figure 2: Sonar footprints and reconstructed sonar beams. (A-H) Sonar footprints of type 2 (A, C, E), type 1 (B, D) and approach signals (F-H) of the call sequence depicted in Figure 1 on the square array. The SPL is color-coded and interpolated between the microphones (black dots) on the array plane. The black “+” represents the center of the array, the black “x” the calculated maximum. (J-O): Reconstructed sonar beams of signals recorded with the square array (J-L) and the chain array (M-O). The SPL is color-coded and indicates the beam shape relative to the beam maximum in the center of a polar plot. The black dots mark the positions of the microphones, the black “x” marks the calculated apparent or real beam maximum. Type 1 signals are depicted in (J) and (M), type 2 signals in (K) and (N), and approach signals in (L) and (O). Note that type 1 signals are directed to the lower part of the array whereas type 2 signals are directed to the upper part.

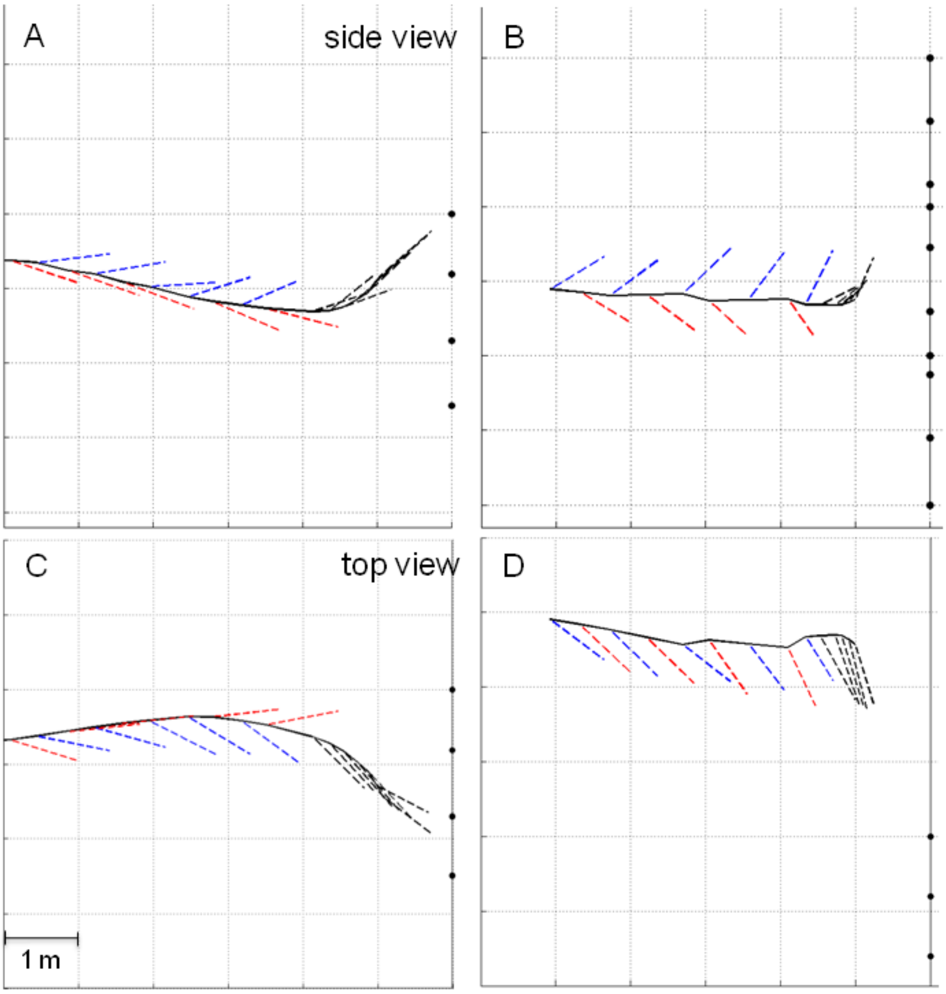

Figure 3: Changes in beam direction in exemplary flights towards the square and the chain array. (A, B) depict side views and (C, D) overhead views of exemplary flights. The black dots represent the microphones array. The 4 m high square array was positioned ~ 1.6 m above ground (A, C) while the 6 m high chain array was positioned about 0.3 m above ground (B, D). The flight paths are depicted as black lines. At each position where a signal was emitted a vector pointing towards the calculated apparent beam maximum on the array indicates the apparent beam direction. The vectors of type 2 signals are depicted in blue and those of type 1 signals in red. Black vectors indicate the direction of approach signals. Note that in the flight depicted in (B, D), the bat passed on the left side of the array. Thus all horizontal beam directions are artifacts pointing to the right side whereas the bat might be facing straight ahead. However, the vertical angles remain unaffected by this offset.

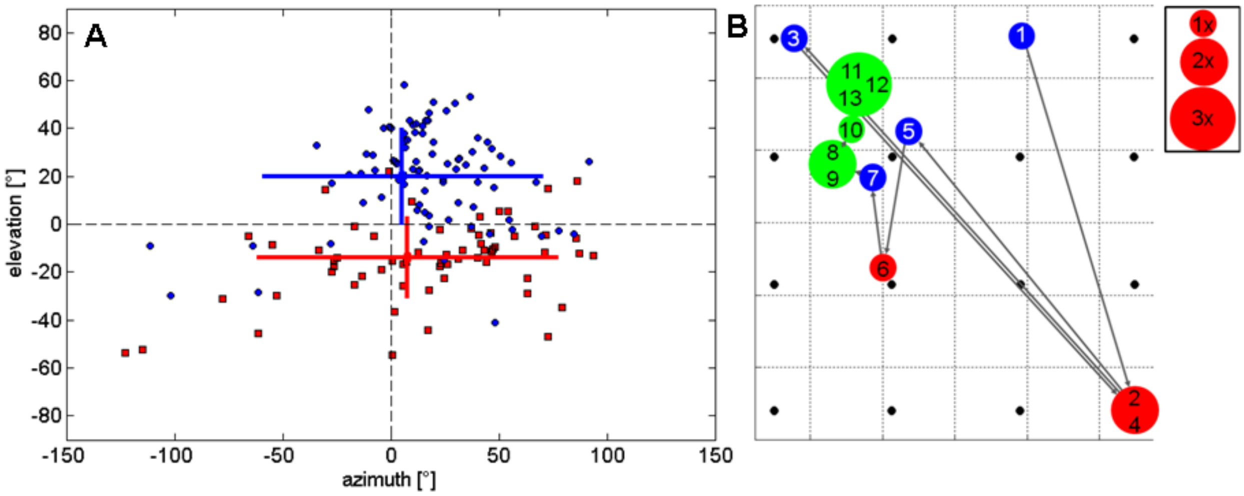

Figure 4: Separation of apparent beam directions of type 1 and type 2 signals. (A) Based on 19 sequences containing 66 type 1 signals (red) and 88 type 2 signals (blue) of bats flying towards the square array, measured angles between the flight path and the calculated apparent beam vector in the vertical (elevation) and horizontal (azimuth) projection plane. The colored bars indicate the respective means and standard deviations. Note the clear separation between signal types. The vectors of type 1 signals are positioned mainly below and those of type 2 signals mainly above flight direction. (B) Pulse-to-pulse path of the calculated apparent or real beam maxima of an exemplary flight on the square array plane with successive numbers showing their order. Type 1 signals are depicted in red, type 2 signals in blue, and approach signals in green. Circles are proportional to the number of calls pointed to this location. The black dots depict the 16 microphones.

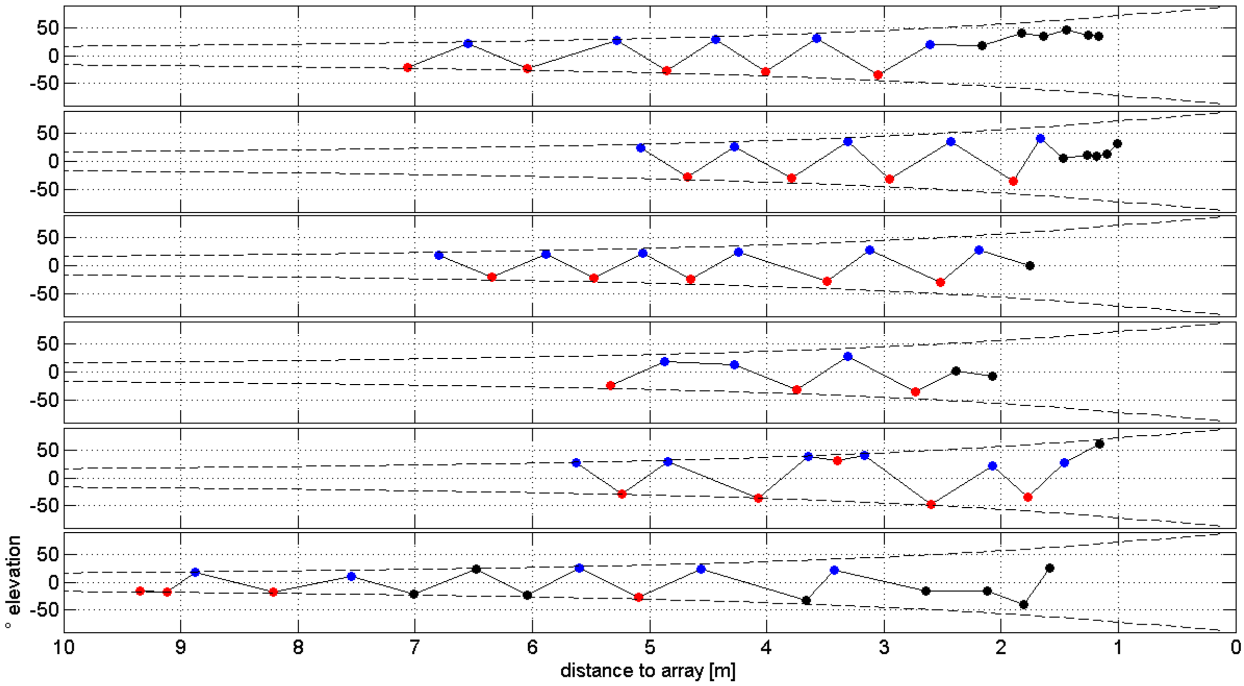

Figure 5: Separation of apparent beam directions of type 1, type 2, and approach signals. 6 exemplary sequences of bats approaching the 6 m high chain array. The position of the apparent beam maximum on the chain array is indicated by the vertical angle between the direction from the bat to the center of the array and the direction of the calculated apparent beam maximum. Differences in color indicate signal types (type 1 in red, type 2 in blue, approach calls in black). The dashed line shows the angle between either limit of the array to its center seen from the bats position and assuming the bat is centered in front of the array.

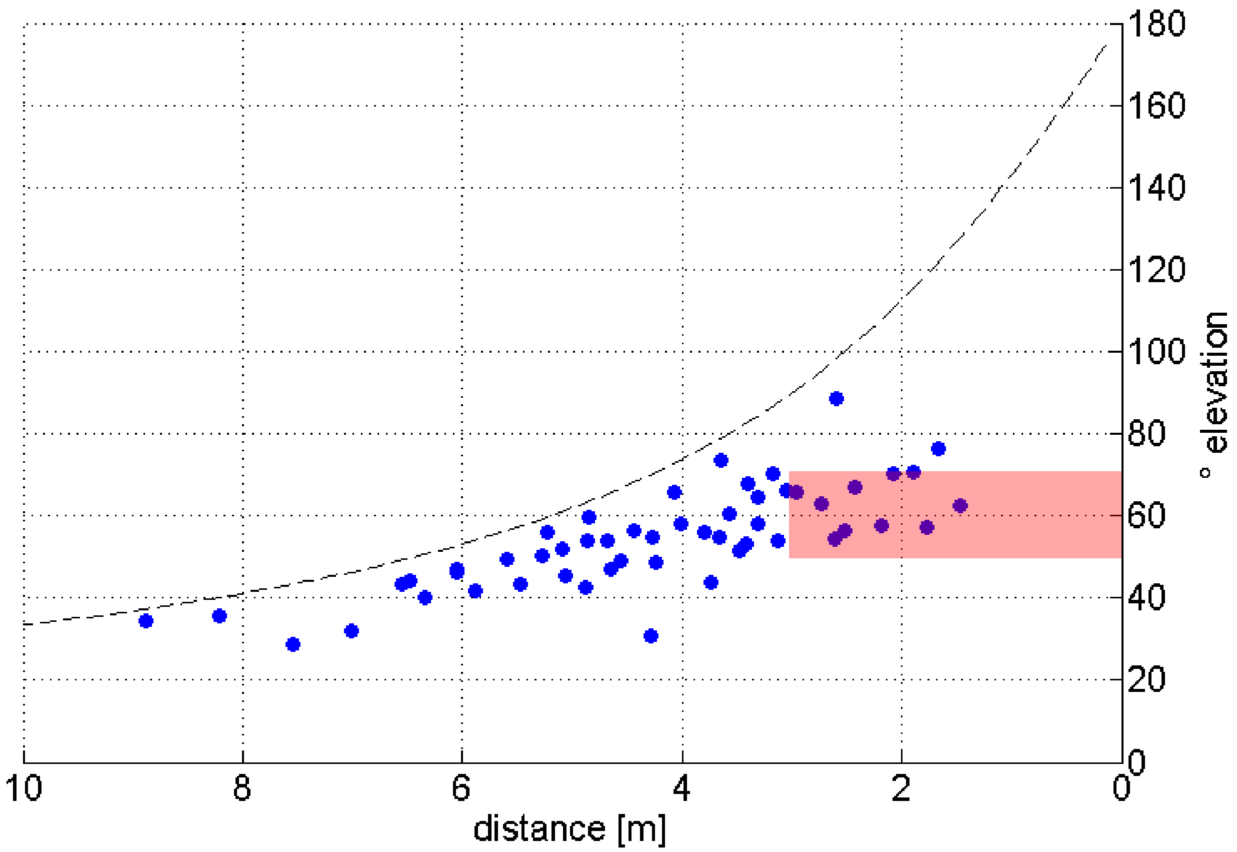

Figure 6: Apparent vertical angles between apparent call directions of type 1 and type 2 signals. The apparent vertical angle between the signal types was determined as sum of the vertical angle for each call type as measured between the direction from the bat to the center of the 6 m high chain array and the direction to the calculated apparent beam maximum (see Figure 5). The dashed line depicts the angle between the upper and lower border of the array as seen from the bats position under the assumption that the bat is centered in front of the array. The values between 10-4 m to the array are limited by this maximal possible angle value. The red rectangle below 3 m marks a range where array height did not limit the measured angles.

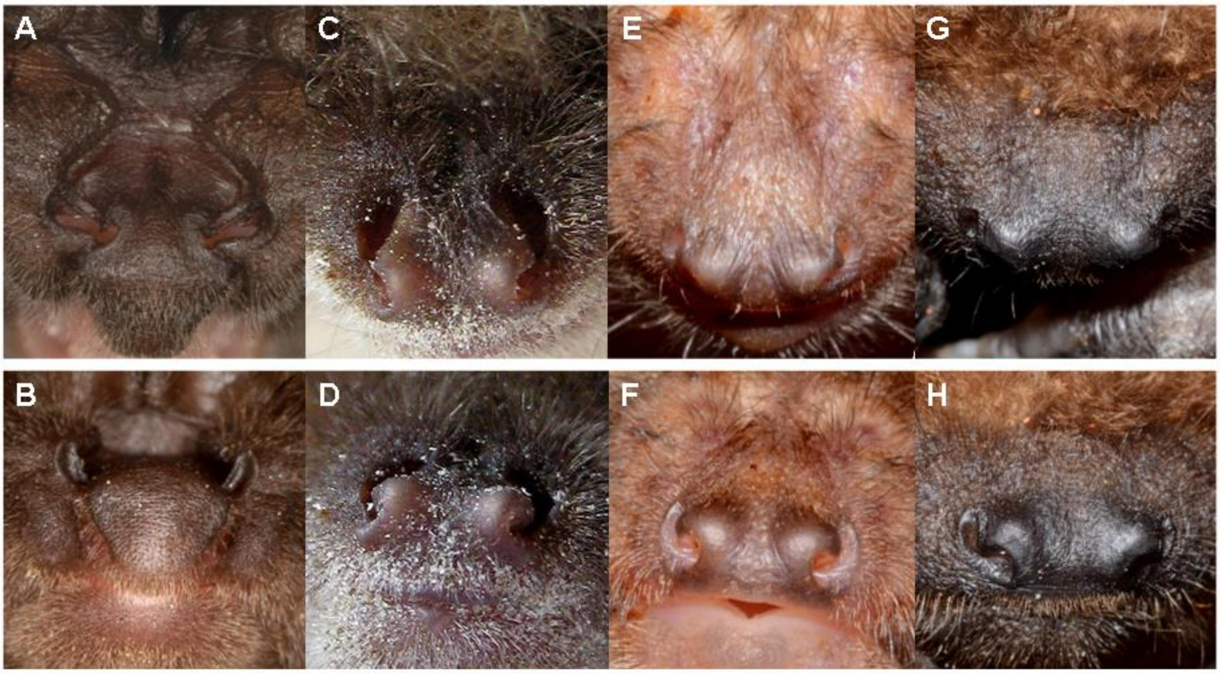

Figure 7: Nostril alignment in four species of Vespertilionid bats.Top view in the upper row and front view in the lower row of the noses of *Barbastella barbastellus* (A, B), *Plecotus auritus* (C, D), *Myotis bechsteinii* (E, F), and *Nyctalus noctula* (G, H). In *M. bechsteinii* and *N. noctula* the nostrils point forward and are not visible from above. In *B. barbastellus* and *P. auritus* the nostrils are turned upward and are therefore clearly visible from above and only partly visible in the front view. *Photo courtesy: (A, B) Christian Dietz; (C, D) Anna-Maria Seibert; (E-H) Laurent Arthur.*

Table 1: Signal parameters of type 1 and type 2 echolocation calls. Parameters of echolocation signals in *Barbastella barbastellus* emitted when flying towards the microphone array. For each sequence only the mean of the contained parameters were used to avoid pseudoreplication.

call type		**type 1**	**type 2**
		(n=86, N=24)	*(n=110, N=23)*
duration [ms]	mean (± SD)	1.7 (± 0.4)	2.4 (± 0.6)
	min	0.9	1.4
	max	2.5	4.7
		(n=19, N=9)	*(n=43, N=7)*
pulse interval [ms] between calls	mean (± SD)	67.9 (± 12.8)	59.3 (± 11.4)
	range	49.0 - 92.1	42.3 - 92.0
		(n=10, N=4)	*(n=31, N=7)*
pulse interval [ms] between types	mean (± SD)	144.0 (± 60.0)	99.8 (± 31.8)
	range	99.9 - 262.5	47.5 - 150.9
		(n=86, N=24)	*(n=110, N=23)*
start freq [kHz]	mean (± SD)	35.9 (± 1.6)	44.4 (± 1.4)
	min	33.1	38.2
	max	43.5	47.5
		(n=86, N=24)	*(n=110, N=23)*
terminal freq [kHz]	mean (± SD)	31.3 (± 1.0)	35.2 (± 2.6)
	min	29.6	26.9
	max	35.2	38.7
		(n=86, N=24)	*(n=110, N=23)*
bandwidth [kHz]	mean (± SD)	4.6 (± 1.2)	9.1 (± 2.5)
	min	2.9	5.9
	max	8.8	16.6
		(n=86, N=24)	*(n=110, N=23)*
peak freq [kHz]	mean (± SD)	33.6 (± 1.5)	40.1 (± 2.2)
	min	31.4	27.4
	max	40.1	42.6
		(n=86, N=24)	*(n=110, N=23)*
apparent source level [dB]	mean (± SD)	80.5 (± 5.5)	82.7 (± 5.0)
	min	70.0	69.6
	max	97.0	96.6
		(n=14, N=8)	*(n=11, N=7)*
source level [dB]	mean (± SD)	83.2 (± 6.7)	82.3 (± 4.1)
	min	70.0	77.1
	max	90.7	87.8

N=number of recorded sequences, n=number of signals used to calculate the corresponding mean.

5.4 Asymmetry and dynamics of a narrow sonar beam in an echolocating harbor porpoise

This paper was published in the Journal of the Acoustical Society of America in 2012. The array hardware was designed by Aarhus University. The amplifier was designed by the electronic workshop of the department of animal physiology, University of Tübingen. Peter Madsen calibrated the hydrophones and played a crucial role in writing the manuscript. Magnus Wahlberg assisted in the experimental design and in the recordings, he provided matlab codes to analyze the data and co-authored the manuscript. Kristian Beedholm provided the matlab code to generate the piston model. The trainers at Fjord og Bealt trained the animal and were crucial during data collection. I recorded the data, performed most of the analysis and co-authored the paper.

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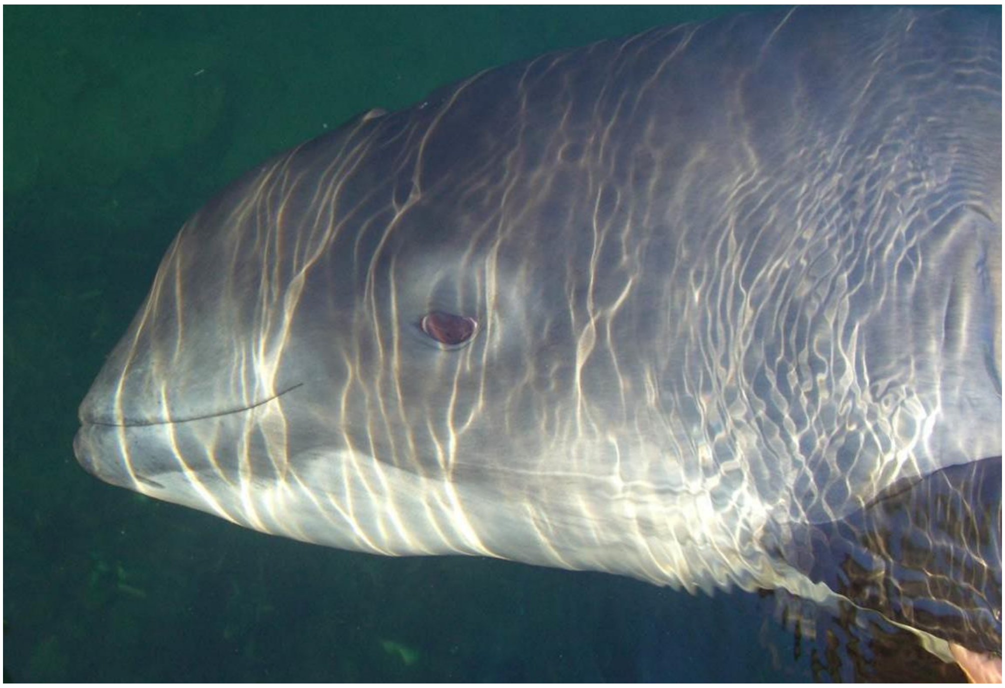

Figure 18: The head of a harbor porpoise. Photo: Jens Koblitz, Fjord og Baelt.

Asymmetry and dynamics of a narrow sonar beam in an echolocating harbor porpoise

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A key component in the operation of a biosonar system is the radiation of sound energy from the sound producing head structures of toothed whales and microbats. The current view involves a fixed transmission aperture by which the beam width can only change via changes in the frequency of radiated clicks. To test that for a porpoise, echolocation clicks were recorded with high angular resolution using a 16 hydrophone array. The beam is narrower than previously reported (DI = 24 dB) and slightly dorso-ventrally compressed (horizontal −3 dB beam width: 13°, vertical −3 dB beam width: 11°). The narrow beam indicates that all smaller toothed whales investigated so far have surprisingly similar beam widths across taxa and habitats. Obtaining high directionality may thus be at least in part an evolutionary factor that led to high centroid frequencies in a group of smaller toothed whales emitting narrow band high frequency clicks. Despite the production of stereotyped narrow band high frequency clicks, changes in the directionality by a few degrees were observed, showing that porpoises can obtain changes in sound radiation.

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I. INTRODUCTION

All microbats (Microchiroptera) and toothed whales (Odontoceti) investigated so far use echolocation as a primary sensory modality for spatial orientation and food acquisition (Griffin, 1958; Au, 1993). When toothed whales echolocate, ultrasonic clicks of very high intensity are produced within the nasal complex by forcing pressurized air past pairs of phonic lips (Cranford *et al*., 1996; Cranford, 2000). The produced clicks propagate through a rostrally placed fatty melon into the water. Returning echoes from objects in the water column are subsequently transmitted to the inner ear through fat bodies in the lower jaw and other regions of the head (Au, 1993; Cranford *et al*., 1996; Ketten, 2000). Successful echolocation of a prey item requires that the returning echo is received with a sufficient signal to noise ratio to allow for detection in the auditory system, calling for high source levels and acute hearing.

The acoustic signals of echolocating toothed whales are emitted in a highly directional beam. The higher the directionality the larger the source level for a given amount of radiated energy, leading to a longer detection range of prey on the acoustic axis for a noise limited situation. In addition, a directional sound beam reduces the clutter and reverberation from, e.g., the surface or bottom. The directionality of echolocation clicks is thus an important parameter for evaluating the performance and evolutionary driving forces of toothed whale biosonar systems (Au, 1993; Madsen and Wahlberg, 2007).

In toothed whales the beam patterns of echolocation clicks have been measured for a number of species, including the bottlenose dolphin, *Tursiops truncatus* and *T. aduncus* (Au *et al*., 1978; Au *et al*., 1986; Au, 1993; Wahlberg *et al*., 2011), the beluga, *Delphinapterus leucas* (Au *et al*., 1987), the false killer whale, *Pseudorca crassidens* (Au *et al*., 1995), the white-beaked dolphin, *Lagenorhynchus albirostris* (Rasmussen *et al*., 2004), the sperm whale, *Physeter macrocephalus* (Mohl *et al*., 2003; Zimmer *et al*., 2005b), Cuvier's beaked whale, *Ziphius cavirostris* (Zimmer *et al*., 2005a), finless porpoise, *Neophocaena phocaenoides* (Akamatsu *et al*., 2005), Peale's dolphin, *Lagenorhynchus*

[a)] Author to whom correspondence should be addressed. Present address: German Oceanographic Museum, Katharinenberg 14-20, 18439 Stralsund, Germany. Electronic mail: jens.koblitz@uni-tuebingen.de *australis* (Kyhn *et al*., 2010), Commerson's dolphin *Cephalorhynchus commersonii* (Kyhn *et al*., 2010) and the harbor porpoise, *Phocoena phocoena* (Au *et al*., 1999) (Table I).

The beam width is either parameterized by the directivity index (DI), expressing the ratio between the source level of a directional and an omnidirectional transducer radiating the same acoustic power, or by the −3 dB (half power) beam width (BW) in degrees. The −3 dB BW is defined as the angle between the directions at which the sound pressure level is reduced by 3 dB to either side of acoustic axis in the horizontal or vertical plane. The measured beams of most species are narrow and similar in beam width, with the −3 dB BW ranging from 6.5° in the beluga to 9–10° in the bottlenose dolphin (Au *et al*., 1987; Au, 1993; Wahlberg *et al*., 2011). The harbor porpoise is an exception in that it is reported to have a wider beam with a −3 dB BW of 16° (Au *et al*., 1999). Most sonar beams described to date are rotational symmetric, but for many of the quoted estimates, the methodologies have not allowed for detection of rotationally asymmetric beams. Only for the false killer whale a lateral compressed beam has been found with a vertical −3 dB BW of 9.7° and a horizontal −3 dB BW width of 6.2° (Au *et al*., 1995).

The width of the sound beam depends on a range of factors, including the frequency content of the radiated signals, the size and morphology of the skull, the melon and the air sacs connecting to the phonic lips and nasal passages (Aroyan *et al*., 1992; Au *et al*., 1995; Cranford, 2000). The beam pattern may be modeled by the one generated by a flat circular piston oscillating in an infinite baffle. This model was introduced by Strother and Mogus (1970) for bats and by Au *et al*. (1978) for toothed whales. Such a model is attractive due to its relative simplicity, and it matches measured beam characteristics fairly well (Au, 1993; Madsen and Wahlberg, 2007; Beedholm and Møhl, 2006). Based on this model, directionality depends solely on the spectrum of the emitted signal and the aperture of the emitter. This allows for computation of what has been coined an equivalent aperture providing the size of a flat piston with the same radiation pattern as that of the animal in question for a given sound. Thus, the directionality of an emitted signal increases when the transducer aperture increases and/or higher frequencies are emitted (Madsen *et al*., 2004; Urick, 1983).

A group of smaller toothed whales, the Phocoenidae, *Cephalorhynchus spp*. dolphins, the pygmy and probably dwarf sperm whales (Kogiidae) all emit narrowband high frequency (NBHF) clicks (Madsen *et al*., 2005). Since directionality depends on the relation between emitter size and frequency, smaller toothed whales using a high frequency emphasis in their signals may at least in part be able to compensate for their size and be able to generate the same

TABLE I. Summary of toothed whale transmission beam pattern measurements.

Species	Horizontal−3 dB BW	Vertical−3 dB BW	Directivity index [dB]	Source
Sperm whale *Physeter macocephalus*	n.a	n.a	27	Mohl *et al*. (2003)
Sperm whale *Physeter macocephalus*	n.a	n.a	26.7	Zimmer *et al*. (2005b)
Cuvier's beaked whale *Ziphius cavirostris*	n.a.	n.a.	>25.	Zimmer *et al*. (2005a)
Beluga *Delphinapterus leucas*	6.5	6.5	32.1	Au *et al*. (1987)
Bottlenose dolphin *Tursiops truncatus*	9.7	10.2	25.8	Au (1993)
Bottlenose dolphin *Tursiops truncatus*	n.a.	n.a.	26.5	Au *et al*. (1986)
Bottlenose dolphin *Tursiops truncatus*	9.8	10	n.a	Au *et al*. (1978)
Bottlenose dolphin *Tursiops truncatus*	9	9	26	Wahlberg *et al*. (2011)
Bottlenose dolphin *Tursiops aduncus*	8	8	29	Wahlberg *et al*. (2011)
False killer whale *Pseudorca crassidens* (Type IV signals)	6.2	9.7	28.5	Au *et al*. (1995); Au *et al*. (1999)
White-beaked dolphin *Lagenorhynchus albirostris*	8	8	29	Rasmussen *et al*. (2004)
Peale's dolphin *Lagenorhynchus australis*	n.a	n.a	25	Kyhn *et al*. (2010)
Commerson's dolphin *Cephalorhynchus commersonii*	n.a	n.a	25	Kyhn *et al*. (2010)
Harbor porpoise *Phocoena phocoena*	16.5	16.5	22.1	Au *et al*. (1999)
Harbor porpoise *Phocoena phocoena*	13.1	10.7	24	this study

directionality as larger species using a lower frequency emphasis. Recent field measurements of two other NBHF species showed that their beam widths are indeed similar to the ones of other smaller toothed whales (Kyhn *et al.*, 2010). A previous study on the harbor porpoise, however, suggests that this NBHF species has the widest beam of all toothed whales investigated (Au *et al.*, 1999).

Moore *et al.* (2008) showed that the bottlenose dolphin can change its beam width and steer its beam (i.e., move the acoustic axis of the beam relative to its body axis) when faced with a task that requires the detection of objects placed off the acoustic axis. A variable beam width would seemingly be advantageous during prey pursuit and could at least partially be caused by shifts in the frequency of the emitted click (Madsen *et al.*, 2004). Recently Jakobsen and Surlykke (2010) have shown that bats widen their beam during the last phase of prey pursuit by lowering the frequency emphasis of their calls. A wider beam allows the bat to track insects at close distances despite escape manoeuvres of the prey. It is not clear how, to what degree and with what purpose toothed whales may be doing the same, and up until the study of Moore *et al.* (2008) beam dynamics of toothed whales have only been considered to relate to changes in frequency (Au *et al.*, 1995; Madsen *et al.*, 2004).

Such lack of insight relates to the fact that most previous studies on the beam characteristics of odontocetes average over many measurements, making it impossible to investigate beam dynamics. In this study we wished to address if a NBHF species, such as a porpoise emitting relatively stereotyped signals, can also change its beam pattern when echolocating.

Using a 16-channel hydrophone array we here report the vertical and horizontal beam pattern of an echolocating harbor porpoise with high angular resolution. We show that the porpoises beam is narrower than previously measured, dorso-ventrally compressed and dynamic.

II. MATERIALS AND METHODS

A. Hydrophone array and recording system

Recordings were made with 16 individually calibrated Reson TC-4013 hydrophones arranged in a plus-shaped array (four arms separated by 90°) with one central hydrophone and three hydrophones on each arm (upwards, downwards, left and right). The hydrophones were located at distances of 10.6, 17.5, and 35.2 cm in each direction from the central hydrophone, corresponding to 3°, 5°, and 10° off center when the clicking porpoise is 2 m away. The three arms extending upwards, left and right, had an additional hydrophone 53.5 cm from the central hydrophone, 15° off center (Fig. 1). The array was made of solid PVC pipes with a diameter of 3 cm. The hydrophones were mounted with their symmetry axes oriented vertically at the end of 7 cm long (1.5 cm diameter) PVC pipes extending from the array frame. In this way any possible weak reflections from the array would arrive more than 94 μs after the direct path, and thus after the main part of the porpoise click. Signals were amplified by 38 dB and filtered using a custom-made 16 channel amplifier and filter, and then simultaneously A/D converted with 16 bit resolution at a sampling rate of 500 kHz per channel (National Instruments PXI-6123). An underwater video camera (Profiline CTV7040) was used to monitor the harbor porpoise movements on station. The signal from the video camera was digitized with a Sony GV-D1000E DV Video Walkman. All 16 channels from the hydrophone and the video recordings were saved in a ring buffer, and after triggering the system the past 4 s of recordings were stored on a laptop computer using a customized version of SIMI Motion (version 7.3 build 269).

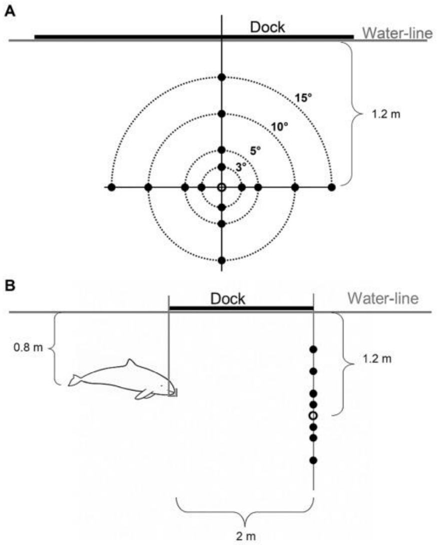

FIG. 1. Recording set-up. (a) Animal's perspective. Degrees are relative to the central hydrophone and when the animal is stationed 2 m in front of the array. Central hydrophone is indicated with an unfilled circle and peripheral hydrophones with filled circles. (b) Side view. Animal was positioned at a depth of 0.8 m and pointing downwards, the central hydrophone was at a depth of 1.2 m.

B. Calibration of the hydrophones

The 16 hydrophones were calibrated in an anechoic tank using a B&K 8105 hydrophone as a transducer and a B&K 8103 hydrophone as the standard. Calibration was made in the frequency range between 110 and 150 kHz, which is the relevant range for harbor porpoise signals. The hydrophones had an average sensitivity of −212 dB re 1V/1μPa at 130 kHz and each one had a flat (within 2 dB) frequency response within the tested frequency range. The difference between the hydrophone of highest and lowest sensitivity was 1.3 dB. To correct for sensitivity differences due to hydrophone attachment or array arrangement, we placed an omnidirectional transducer (HS 150, Sonar Research & Development Ltd, Beverley, UK) 2 or 4 meters in front of the array on each recording day and emitted porpoise-like clicks (15 cycle pulses at 130 kHz). These signals were then used to normalize all hydrophone sensitivities with respect to the center hydrophone during post processing.

C. Animal and training

The trials were made at Fjord&Bælt in Kerteminde, Denmark, where four harbor porpoises are housed in an outdoor facility. The facility is connected to the harbor of Kerteminde through nets. Wind and current conditions during recordings were very calm, resulting in good underwater visibility. One 12 year old male harbor porpoise (FBC-01) was trained to station voluntarily in a u-shaped chin rest 2 m in front of the hydrophone array with the tip of the rostrum touching a small square PVC target. The depth of the central hydrophone of the array was 1.2 m, and the depth of the target was 0.8 m, when the animal was on station it was pointing downwards. The correct position of the animal is referred to as "on station" hereafter. The animal was sent to station by the trainers and observed visually from above by the trainers, and with an underwater video camera, attached to the array and facing the animal, by the experimenter. The visual observations ensured that the animal was on station during the recordings. No behavioral tasks were required from the animal during the recordings, but it nevertheless echolocated towards the hydrophone wall.

D. Analysis

Data analysis was made using routines written in MATLAB 7.0 (MathWorks, Inc.). Clicks were detected using a threshold based peak detector. Only clicks with a source level of 132 dB re 1 μPa (peak to peak) or higher were analyzed. The source level is defined as the sound intensity back-calculated to 1 m range on the acoustic axis (Urick, 1983). The intensity in any other direction of the sound source we denote the apparent source level (ASL) sensu Møhl *et al*. (2003). The apparent source level in units of peak to peak was measured for each detected click of the 16 channels. The clicks with a maximum apparent source level on the central hydrophone (after being compensated for the difference in sensitivity between the receivers) were regarded as being recorded on the acoustic axis and saved for further analysis. The beam patterns were plotted as the apparent source level as a function of the angle relative to on axis. A piston modeled beam pattern was fitted to the measured beam patterns using the equations in Au (1993), and the directionality index was estimated using the equation of Møhl *et al*. (2003).

III. RESULTS

When stationed in the U-shaped chin rest two meters in front of the array, the porpoise mostly emitted clicks with a high repetition rate and low source level (SL) interspersed with a few clicks emitted at lower repetition rates and higher SL. At high repetition rates, the inter-click intervals varied between 2.5 and 15 ms. Out of a total number of 74 trials, two trials resulted in recordings of on-axis click trains which are characterized by consecutive signals with maximum sound level on the central hydrophone. In these clicks a maximum variation in source level of 8 dB (132–140 dB re $1\mu\text{Pa}_{\text{pp}}$) was measured. The average click (N= 464) signal waveforms did not differ when recorded off-axis at the angles covered here [Figs. 2(a) and 3] and the averaged spectra [Fig. 2(b)] show that the spectral composition is very similar in the forward direction out to 15° off axis.

For each of the on-axis clicks the apparent source level (ASL) measurements with the nine hydrophones in the horizontal plane and the eight hydrophones in the vertical plane were used to determine a horizontal and a vertical beam. Overlaying the beam patterns of all 464 on-axis clicks show variations of the ASL relative to the on-axis SL at each hydrophone. This variation increases with increasing off-axis angle [Figs. 4(a), 4(c), 5(a) and 5(c)]. Part of the observed variations can be explained by the spatial sampling. The hydrophone arrangement with the first ring of hydrophones three degrees from the central hydrophone led to clicks directed up to 1.5° away from the central hydrophone to be classified as on-axis. Since the animal was not fixed but free to move, movements in the range of $+/-1.5°$ increased the variability of the beam. To account for these variations we made cubic spline interpolations over the nine beam measurement points in the horizontal plane and eight in the vertical plane. The interpolated beam maximum was, as expected, not exactly on the central hydrophone but often off by $+/-1.5°$ [Figs. 6(a) and 6(c)]. We assumed that this

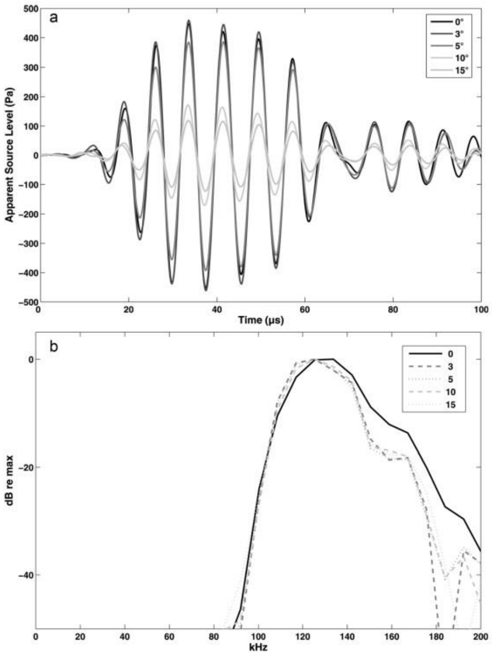

FIG. 2. (a) Averages of 464 harbor porpoise clicks recorded at five different angles (0°, 3°, 5°, 10°, and 15°) relative on axis. Sampling frequency was 500 kHz and signals were interpolated with a factor of 10. (b) Mean normalized spectra of 464 signals from Fig. 2(a). FFT size 600, Hanning window, sampling frequency 500 kHz.

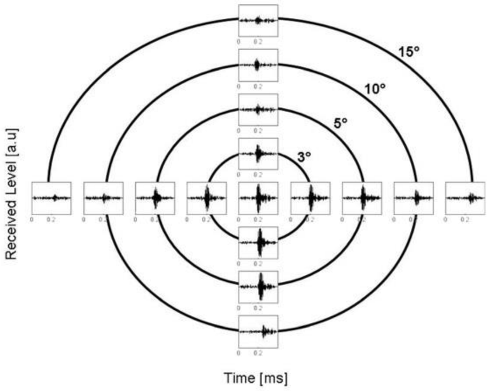

FIG. 3. Received harbor porpoise echolocation signals on each hydrophone of the array, as seen from the animal's perspective at 2 m range.

jitter was caused by small variations of the direction of the animal's acoustic axis and we accounted for these minor movements of the animal in the following way: For each click, the vertical and horizontal beam patterns were interpolated with cubic spline interpolation and then aligned to the interpolated maximum [Figs. 4(b), 5(b) and 6(b), 6(d)]. A composite beam pattern was made by averaging over these interpolated and aligned 464 beam patterns [Figs. 4(d) and 5(d)].

After accounting for the beam jitter within ±1.5 the click amplitudes still showed some variation which increased with increasing off-axis angle. Additionally, the horizontal and the vertical beam patterns were not symmetrical around the acoustical axis [Figs. 4(d) and 5(d)].

The average horizontal one-sided -3 dB beam width was 6.2° (std: 0.84°) to the left and 6.9° (std: 1.1°) to the right of the acoustic axis. The average one-sided vertical -3 dB beam width was 4.8° (std: 1.3°) above and 5.8° (std:1.1°) below the acoustic axis. The average -3 dB horizontal beam width was thus 13.1° (std: 1.2°) in the horizontal plane and 10.7° (std: 0.99°) in the vertical plane, resulting in a slight, but significant dorso-ventrally compressed beam (paired t test, $p<0.001$, d.f.= 463).

The porpoise beam width was not fixed but showed some variation: While the -3 dB beam width to the left of the acoustic axis was constant for clicks 48–77, an increase of 2° and more could be seen on the right side of the beam (Fig. 6(b)). This increase exceeds 2 standard deviations.

The click amplitudes measured in the vertical plane with the upper 15° hydrophone indicate the presence of a sidelobe (Fig. 5). We have had no hydrophone at the lower 15° position so that we can make no statement on the vertical beam shape below 10°. Within the $+/-15$° measuring range, no side lobe in the horizontal beam pattern was indicated (Fig. 4).

The best fit of the data to a modeled flat, circular piston emitting a porpoise click in an infinite baffle was obtained with a horizontal equivalent aperture of 6.5 cm and a vertical equivalent aperture of 8.3 cm (Fig. 7). The variability seen in the data could be explained by aperture changes from 5.5 to 7.4 cm in the horizontal plane and from 7.1 to 9.6 cm in the vertical plane.

IV. DISCUSSION

A. Toothed whales have similar beam widths across taxa

We show here that the harbor porpoise echolocation beam is narrower than previously reported and thus not as different from other toothed whales as previously published data would indicate. Au *et al*. (1999) reported that the harbor porpoise beam is the broadest of all toothed whales investigated so far with a -3 dB beam width of 16° in both the vertical and horizontal plane, corresponding to a DI of 22.1 dB.

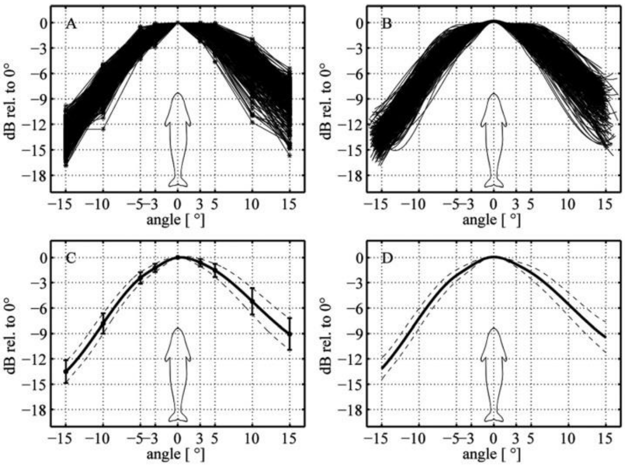

FIG. 4. (a) Horizontal beam pattern for 464 on-axis clicks of a harbor porpoise. (b) Interpolated and shifted horizontal beam pattern for all on-axis clicks. (c) Averaged horizontal beam pattern. Error bars show mean and 1 standard deviation of the measurements for each receiver. Black solid line is the averaged interpolated beam pattern. One standard deviation of all interpolated beams is depicted by the black dashed line. (d) Averaged horizontal beam pattern corrected for scanning movements. Dashed lines show 1 standard deviation. By correcting for the scanning, the standard deviation is reduced.

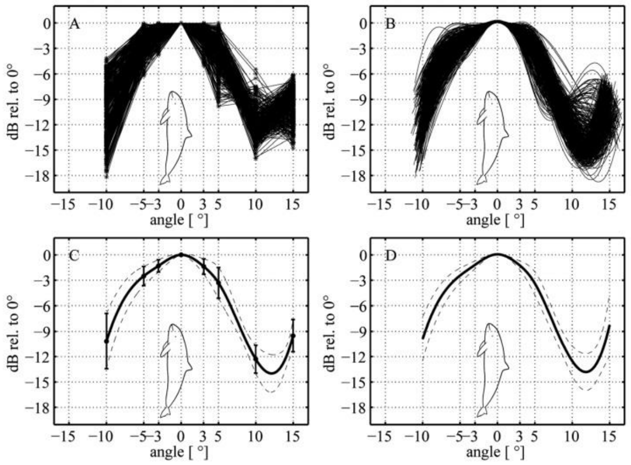

FIG. 5. (a) Vertical beam pattern for 464 on-axis clicks of a harbor porpoise. (b) Interpolated and shifted vertical beam pattern for all on-axis clicks. (c) Averaged vertical beam pattern. Error bars show mean and one standard deviation of the measurements for each receiver. Black solid line is the averaged interpolated beam pattern. The 1 standard deviation of all interpolated beams is depicted by the black dashed line. (d) Averaged vertical beam pattern corrected for scanning movements. Dashed lines show standard deviation. By correcting for the scanning, the standard deviation is reduced.

We found the beam to be narrower, especially in the vertical plane with a −3 dB beam width of 10.7° but also in the horizontal plane with a −3 dB BW of 13.1°, corresponding to a DI of 24 [derived using the approximate relationship, $V_{3dB} \approx 185° \times 10^{(-DI/20)}$ (Lurton, 2002; Zimmer *et al.*, 2005a)]. This indicates that the beam is in fact only slightly broader than the beam of most other smaller toothed whales (Au, 1993; Wahlberg *et al.*, 2011; Kyhn *et al.*, 2010). This is lending weight to the contention that toothed whale sonars have been faced with an evolutionary driving force to achieve high directionality in order to increase the source level in the forward direction and to reduce reflections from the periphery. Reported beam width in all species ranges from 6.5° for the beluga (Au *et al.*, 1987) to 13.1° in the horizontal plane of the harbor porpoises, corresponding to a DI difference of approximately 9 dB. Similar echolocation beam directionalities across species of various sizes have been recently discovered in bats. Five species ranging in size from 8 to 26 g and in the main frequency emitted from 20–55 kHz, all have strikingly similar directionalities, since small bats echolocate at higher frequencies (Jakobsen, 2010). It is intriguing that species living in extremely different acoustic environments all seem to have ended up with similar beam patterns. This seems to hold true both for across bat species as well as across smaller toothed whales.

The use of high frequencies around 130 kHz generates a narrow transmission beam for the many small NBHF species. Simultaneously, echolocation clicks at these high frequencies make NBHF species inaudible to a major predator, the killer whale (*Orcinus orca*). Whether predation or obtaining high directionality (or both) is the major reason for the use of high frequencies is currently not known. The present data strongly indicate that all smaller toothed whales investigated have surprisingly similar beam widths across taxa and habitats, and that the achievement of a narrow beam therefore is at least in part responsible for the high centroid frequencies of the small NBHF species (Kyhn *et al.*, 2010). The differences in beam width between this study and the one by Au *et al.* (1999) can

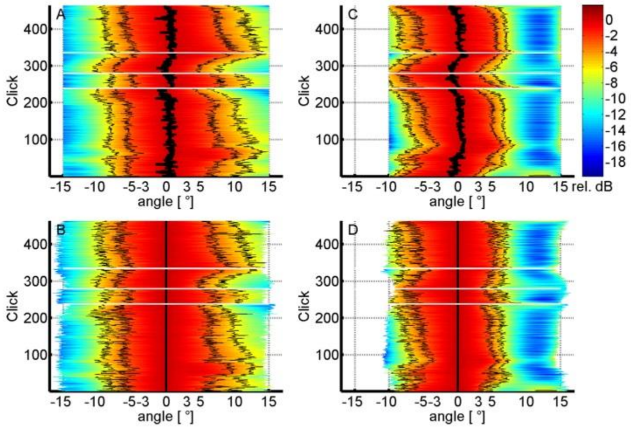

FIG. 6. (Color online) (a) Interpolated horizontal beam pattern for 464 harbor porpoise clicks (click # on *y* axis). Fat black line shows direction of interpolated maximum, thin black lines −3 dB and -6 dB BW. White horizontal lines represent beginning of new on-axis click train. The animal's beam is on average 0.5° directed to the right. (b) Beam patterns shifted so that direction of maximal intensity is at 0°, i.e., accounting for the scanning movements of the animal. (c) Interpolated vertical beam pattern for each click (click # on *y* axis). Fat black line shows direction of interpolated maximum, thin black lines −3 dB and −6 dB BW. White horizontal lines represent beginning of new on-axis click train. Again the beam is often more off-axis before and after it is considered to be on-axis. (d) Beam patterns shifted so that direction of maximal intensity is at 0°, i.e., accounting for the scanning movements of the animal.

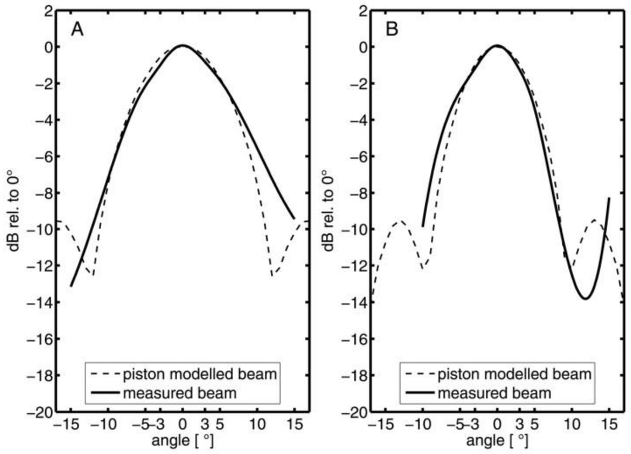

FIG. 7. The measured harbor porpoise transmission beam pattern compared to a modeled beam pattern using a porpoise click emitted by an oval piston with an aperture of 6.5 cm in the (a) horizontal and 8.3 cm in the (b) vertical plane.

possibly be explained by a number of methodological differences. By using more receivers in each plane we achieve a higher angular resolution. The use of a linear array (vertical or horizontal) did not make it possible for Au *et al*. (1999) to determine whether the acoustic axis of the beam is centered in both dimensions simultaneously without using a series of other criteria (Madsen and Wahlberg, 2007). Moreover, by using a hoop, the position of the animal might have varied between trials resulting in a larger beam width when averaging. It is extremely unlikely, however, that a varying range between animal and array could have been large enough to significantly affect the beam width measurements. By measuring the beam in both dimensions simultaneously we ensured that we recorded the beam on-axis and only analyzed clicks where the ASL was highest on the central hydrophone (i.e., ASL = SL). The angular distance between the central hydrophone and the first ring of hydrophones surrounding was 3° [Fig. 1(a)]. Thus clicks directed up to 1.5° off-axis in any direction could be classified as on-axis, clicks that were directed further away from the center were not considered for further analysis. This very strict criterion allows us to reduce the apparent variability in the beam width and pattern measurements compared to previous studies. In the subsequent analysis we measured the vertical and horizontal −3 dB beam width for each click simultaneously and then averaged the beam width for each dimension, in comparison to averaging over measurements at one receiver and then computing a beam width from the composite beam pattern (Au *et al*., 1987; Au *et al*., 1999). In addition, we cannot exclude that the different results between us and those of Au *et al*. (1999) could be due to individual differences between the animals investigated or the context in which they were recorded. For example, a larger transmitting aperture will lead to a narrower beam for the same radiated frequency. The animal under investigation here was 12 years old whereas the animal studied by Au and colleagues was younger and smaller (Au *et al*., 1999). In fact, differences in beam width have been found when looking at different individuals of bottlenose dolphins. However, these differences were on the order of less than 1° (Au, 1993; Au *et al*., 1978), and it is therefore unlikely that individual differences alone resulted in a 6° narrower beam width.

B. The beam is slightly dorso-ventrally compressed

The harbor porpoise echolocation beam is not rotational symmetric as previously suggested (Au *et al*., 1999), but rather dorso-ventrally compressed. Most studies on toothed whales beam patterns, including the harbor porpoise, report or assume that the beam had the same width in the vertical and in the horizontal plane (Au *et al*., 1978; Au, 1980; Au *et al*., 1987; Au *et al*., 1999; Zimmer *et al*., 2005b). Our results show a slightly broader beam in the horizontal plane than in the vertical plane. That raises the question if such a difference is the result of a functional driving force or simply a passive consequence of the functional anatomy of the porpoise head. It may be speculated that it is an evolutionary advantage for a shallow water forager such as the harbor porpoise to have a dorso-ventrally compressed beam. Since porpoises mainly swim with their dorsal side oriented upwards (Akamatsu *et al*., 2010), this beam shape would lead to a reduction of especially bottom reflections while still allowing a wider beam in the horizontal plane. By this adaptation, the amount of reverberation from the sea bed could be reduced while still ensonifying a reasonably large volume of water in search for prey. The only other species for which a non-rotational symmetric beam was reported was the false killer whale with a beam narrower in the horizontal (−3 dB BW of 6.2°) than in the vertical plane (−3 dB BW of 9.7°) (Au *et al*., 1995). This species, an open water forager, will not have an obvious advantage from reducing bottom reflections. The differences in beam shape between the harbor porpoise and false killer whale, if not due to differences in methodology, might be the result of different evolutionary factors acting upon the sonar systems of the two species. Alternatively, the differences may be too small to have functional significance and just be the passive result of other driving forces acting on the functional head morphology of these echolocators. While the bilateral symmetry is high in porpoises, the configuration of reflective bones and air sacs are quite different in the vertical plane (Huggenberger *et al.*, 2009) and can possibly explain the observed differences.

C. Beam is not mirror-symmetric in either plane

The beam is not symmetric, neither in the vertical nor the horizontal plane. In the vertical plane, a prominent dorsal side lobe is present. The vertical main lobe of the beam is narrower in the dorsal (−3 dB BW: 4.8°) than in the ventral (−3 dB BW: 5.9°) direction. The asymmetry of the beam increases further off-axis (−6 dB BW dorsal: 6.5° vs ventral: 8.2°). Recently, a frequency dependent two lobed echolocation beam was described for the bottlenose dolphin (Starkhammar *et al.*, 2011). A downwards projected lobe contained energy between 20 and 70 kHz and a slightly upwards projected lobe contained energy at frequencies between 30 and 80 kHz. The observed asymmetry in the harbor porpoise, however, is most likely due to dorso-ventral head asymmetry since the different lobes had the same frequency content. The right half of the −3 dB horizontal beam width is slightly (0.7° or 11%) wider than the left half, again with increasing asymmetry further away from the acoustic axis (−6 dB BW left: 9.1° vs right: 10.6°). The small horizontal asymmetry, although statistically significant, does presumably not have any biological relevance but could be caused by the fact that porpoises are clicking primarily with the right pair of phonic lips (Madsen *et al.*, 2010). Simultaneous measurements of the clicking intensity on the right and left side of the melon generally show more intense radiation towards the right than towards the left side (Au *et al.*, 2010; Madsen *et al.*, 2010). Horizontally asymmetric sonar beams of toothed whales have not been discussed to date but it is seen in the composite beam patterns of the false killer whale (Au *et al.*, 1995). In this species, the right side of the beam seems to be wider than the left side, similar to what is observed in the harbor porpoise. This could hint to single source right-handed click production in the false killer whale as well.

D. Beam pattern variation

Measuring the beam pattern of single clicks in both the vertical and horizontal simultaneously and only including on-axis clicks based on a rigorous criteria allows us to study the beam pattern and the variation in great detail. Most previous studies averaged over many clicks (Au *et al.*, 1978; Au *et al.*, 1986; Au *et al.*, 1999; Rasmussen *et al.*, 2004), leading to an average beam pattern with often large standard deviations, which were at least in part caused by animal movements; hence, beam dynamic could not be addressed in most studies due to averaging. Here we can in fact demonstrate variation in the beam pattern of 464 on-axis harbor porpoise clicks. This indicates that the porpoise is able to produce a dynamic beam, since this variation is not seen when analyzing recordings from a directional transducer. The observed variation in the range of 2° is small compared to the beam dynamics of a bottlenose dolphin that was able to vary its vertical beam width from 10 to 29° and its horizontal beam width from 15 to 40° (Moore *et al.*, 2008). In the study by Moore *et al.* (2008), the bottlenose dolphin was faced with an angular target detection task, where a wider and dynamic beam would be advantageous. In our experiment, the harbor porpoise was not faced with any task, so future studies will have to test if this species is also able to produce a beam as dynamic as the bottlenose dolphin's beam. Bats widen their beam when they close in on their prey by lowering the emission frequency, thus reducing the chance of the prey to escape by evasive manoeuvres (Jakobsen and Surlykke, 2010). Also the dynamics of the bottlenose dolphin's beam were in part caused by frequency shifts, the lower the peak frequency the wider the beam in the horizontal (Moore *et al.*, 2008). Since the harbor porpoise did not change the frequency content of its clicks, the dynamic beam in harbor porpoises are based in conformation changes in the transmitting structures such as melon movements, or changes of the reflective structures in the forms of air sac shapes and volumes. The air sacs might have the function of an inner noseleaf similar to the noseleaf of bats (Zhuang and Muller, 2006) that influences the directionality of the sonar beam, since sound is reflected at the borders between tissue and air due impedance differences. Further, three muscles are attached to the tissue surrounding the fatty melon, which is acting as an acoustic wave guide (Au *et al.*, 2006; Huggenberger *et al.*, 2009; Madsen *et al.*, 2010). These muscles can change the shape of the melon and thus perhaps change sound transmission properties and eventually beam shape. Changes in melon size have been reported for this species (Miller, 2010) and were observed during experimental sessions, making it a likely cause for the observed beam dynamics.

E. Relation to the piston model

Most studies used a flat circular piston to model toothed whale echolocation beam patterns, except Au *et al.* (1995) for the false killer whale. The directional properties for this species was best modeled using a planar rectangular transducer of the size 10.1 × 16.1 cm (Au *et al.*, 1995). The best fit to the average beam pattern of the harbor porpoise in this study was obtained assuming a flat oval piston in an infinite baffle emitting a porpoise click with a horizontal aperture of 6.5 cm and a vertical aperture of 8.3 cm.

The model fits the measured horizontal and vertical beam over a 15° range on either side of the acoustic axis very well. However, the measured dorsal side lobe does not match the piston model, which could be due to the dynamics of single clicks or simply because the porpoise sound emission system cannot be modeled perfectly with a piston. The variability seen in the data could be explained by effective size changes of the emitter (i.e., the melon) through muscle contraction in the range 5.5 to 7.4 cm in the horizontal plane and from 7.1 to 9.6 cm in the vertical plane. Thus while the flat piston model is a good first approximation for beam pattern modeling in echolocating toothed whales, it cannot accommodate the beam dynamics demonstrated here for constant centroid frequencies.

F. Conclusion

All smaller species of toothed whales produce echolocation beams with surprisingly similar high directionality indices around 24 dB (Au, 1993; Kyhn *et al*., 2010), suggesting that sonar needs at least in part seem to have determine the high centroid frequencies of the small NBHF species (Kyhn *et al*., 2010). The harbor porpoise has a slightly dorsoventrally compressed beam which might be an adaptation for this shallow water species where bottom reflections are reduced while the search volume in the horizontal is kept large. Horizontal beam asymmetry supports recent findings, showing that harbor porpoises click with the right pair of phonic lips, whereas vertical echolocation beam asymmetry is probably due to dorso-vental head asymmetry. The dynamics in the porpoise's sonar beam are possibly due to changes of the melon shape, emphasizing that sound radiation from toothed whale nasal complexes cannot be modeled using a flat piston with a fixed aperture. Thus, even with very stereotyped NBHF clicks, porpoises can change their sound transmission to aid biosonar based tracking of prey targets.

ACKNOWLEDGMENTS

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5.5 Single click echolocation beam widths and source levels of three foraging porpoises

Manuscript in preparation for submission to the Journal of Experimental Biology

The array hardware was designed by the mechanical workshop at the University of Tübingen. The same recording hardware as in Koblitz et al., 2012 was used. The trainers at Fjord og Bealt trained the three animals and played a crucial role during data collection. Magnus Wahlberg taught acoustic localization techniques, assisted during the recording sessions, discussed the data and provided valuable comments on the manuscript. I recorded the data, performed the analysis and wrote the manuscript.

Jens C. Koblitz

To be populated with more authors. Significant assistance in experimental design, recordings and analysis was provided by Magnus Wahlberg, Peter Stilz and Hans-Ulrich Schnitzler

Figure 19: A harbor porpoise surfacing in the Fjord og Baelt, Kerteminde. Photo: Jens Koblitz, Fjord og Baelt.

I. Abstract

Three free ranging porpoises capturing prey were recorded with a large 2D planar 15 hydrophone array. The animal’s position at click emission was determined by acoustic localizations and differences in the received levels at the hydrophones were used to measure single click sonarbeam widths. All three individuals emit narrow forward oriented sonarbeams with a – 3 dB beam width between 10.8° and 11.8°. As the animals approached the prey item, the inter-click-interval was reduced from 30-40 ms to 20-30 ms, however the beam width did not change. Confirming the narrow sonarbeam in freely moving porpoises lends weight to the argument that all smaller toothed whales emit their clicks with similar directionality. Smaller animals compensate the lack in emitter size by utilizing high frequencies and achieve the same directionality as larger animals with lower frequencies. Two animals reduced the intensity of the clicks by 10 dB per halving of distance whereas one animal did not show a target distance dependent intensity reduction. The direction of signal emissions varied, especially in the horizontal plane extensive scanning was observed.

II. Introduction

Toothed whales emit echolocation clicks for orientation and foraging in a narrow, forward directed beam (Au, 1993; Madsen and Surlykke, 2013). Sonar parameters under investigation were the inter-click-interval (ICI, the inverse of the click repetition rate), the frequency spectrum, the intensity and the directionality of the emitted clicks. All parameters except for the first need to be measured on the acoustic axis, the direction of signal emission. The spectral composition of clicks deteriorates when measured off-axis, high frequency components decrease in amplitude and spectral notches appear (Au, 1993; Rasmussen et al., submitted; Wahlberg et al., 2011b). When measuring the intensity at an unknown angle relative to the acoustic axis, it is referred to as the apparent source level (ASL) and can underestimate the real source level (SL) on the acoustic axis (Møhl et al., 2000). Studying these signal parameters, especially directionality has been made possible by the use of multi receiver arrays.

The **ICI** has been used to define different behavioral stages in echolocating bats, the search, the approach, and the terminal phase (Griffin et al., 1960). The Griffin model has recently been adapted to describe the echolocation behavior of toothed whales, which similar to bats produce a terminal phase (DeRuiter et al., 2009; Verfuss et al., 2009), however according to this model the approach phase is lacking in some toothed whale species and has been adapted (Madsen et al., 2013).

Source levels of porpoises recorded in the wild exceed those measured of animals in captivity (Kyhn et al., 2013; Villadsgaard et al., 2007) and can reach 205·dB peak-peak re 1 µPa at 1 m. A target range dependent reduction of the SL has been described in detail for numerous species (Au and Benoit-Bird, 2003; Jensen et al., 2009). The intensity is reduced by approx. 6 dB per halving of distance [20*log(distance)], leading to constant echo amplitude at target but not at the echolocator when approaching a small prey item. This target range dependent SL reduction has been studied using the same three animals under investigation here and was found to be approximately 20*log(distance) (Atem et al., 2009; Wisniewska et al., 2012).

The beam width or **directionality** defines the acoustic field of view and in part determines the peripheral perception of objects. The directionality or angular opening of the so called sonarbeam is defined by two physical properties, the wavelength of the emitted signal and the aperture of the transmitter. As the speed of sound is externally determined, the wavelength can only be influenced by the frequency content of the emitted signal. The directionality of different odontocete species has mainly been measured of stationary animals which are echolocating at a linear array of hydrophones (Au et al., 2012; Au et al., 1978; Au et al., 1999; Au et al., 1986; Au et al., 1995; Au et al., 1987; Branstetter et al., 2012). Measuring the beam width of a beam that is not pointed directly at the linear will result in an under determination of the beam width. Some post processing methods have been used to reduce the influence of this error on beam and SL measurements (Madsen and Wahlberg, 2007). The directionality is parameterized as the -3 dB beam width (BW), and defined as the angle between the directions at which the intensity is halved compared to the on-axis intensity. The measured -3 dB BW of sonar beams ranges from 6.5° in the beluga (Au et al., 1987) to 16° in porpoises (Au et al., 1999). The beam width of harbor porpoises, being one of the smallest toothed whale, has thus been measured to be the widest of all toothed whales investigated (Au et al., 1999). Recently another study measured the beam width of another individual and found the beam width to be comparable to other smaller odontocetes (Koblitz et al., 2012). These differences could be due to individual differences of the two animals or differences in methodology. Only recently two dimensional arrays have been used to measure the beam width of stationary animals (Koblitz et al., 2012; Moore et al., 2008) allowing the simultaneous measurement of vertical and horizontal beam width. In addition beam measurements of free ranging odontocetes have been obtained from naïve and unconditioned animals in the wild, however mostly with a vertical linear array, having the same limitations as noted above (Jensen et al., 2013; Kyhn et al., 2010; Kyhn et al., 2013; Wahlberg et al., 2011a; Wahlberg et al., 2011b).

Variation in beam width and **direction of click emission** relative to the body axis have been found for a bottlenose dolphin stationed in front of a large 2D hydrophone array. The animal varied the -3 dB BW from 15° to 40° and the direction of signal emission by up to 18°. The increase in directionality has recently been confirmed in freely moving porpoises. The -3 dB BW increased from 10° when clicks were emitted at an ICI greater than 13 ms to 15-20° during the buzz when clicks were emitted at an ICI of less than 13 ms (Wisniewska et al., in prep).

Here we apply a novel design to measure single click beam widths of three free swimming porpoises using a two dimensional hydrophone array. We investigate whether the beam width is as narrow as recently published, if the beam width shows variation when the animal is emitting clicks at a regular ICI and if there is individual variation of the beam width. In addition we perform accurate source level measurements.

III. Material and Methods

Animal housing and training

Three harbor porpoises (Eigil: male, 42 kg, ~12 years old; Freja: female, 59 kg, ~12 years old; Sif: 48 kg, ~5 years old) were kept in a semi-natural outdoor pool in Kerteminde, Denmark. Animals were trained to find and consume dead fish (sprat and herring) thrown into the holding pool. All animals were in addition trained to wear silicone suction cups to cover their eyes and eliminate vision as a sensory modality.

Recording system

15 Reson TC-4013-5 hydrophones were arranged in 4 columns and 4 rows, with a horizontal and vertical spacing of 0.5 m. Hydrophones were attached to 10 cm long solid PVC pipes extending away from a frame also made of PVC pipes to avoid echoes. The received signals were amplified and high pass filtered using a custom made amplifier. Two National Instruments 6123 DAQ cards with a simultaneous sampling rate of 500 kHz per channel and 16 bit converted the signal from analog to digital. Each recording made was 6 seconds long covering most or all of the animals approach. An underwater camera was mounted behind the array facing towards the approaching porpoises. The video recordings were synchronized with the audio recordings. Recordings were monitored and triggered using the software Simi Motion.

Data recording

One animal was at one end of the pool with a trainer and given the signal to approach the hydrophone array. Simultaneously a fish was thrown approximately one meter in front of the array, in addition a target stick was splashed onto the water to provide an extra cue. The porpoise then swam towards the prey item, the capture success was monitored with the underwater camera.

Data analysis

Only recordings were selected where acoustic localization and beam measurements were possible for the major part of the approach, i.e. where the animal mainly echolocated towards the hydrophone array. A total of 16 approach sequences (4-7 trials per animal) were chosen for subsequent analysis, resulting in 1167 clicks.

Localizing the animal

Of the 15 recorded channels, subsets of receivers with a good signal to noise ratio were used to compute the time of arrival difference between one template receiver and each of the subset receivers. The resulting swim paths for each subset were screened for outliers and smoothed. In the consequent analysis clicks with an up to 6 dB lower received level were also considered. The position at click emission was interpolated based on the localized positions (anchor points) of the animal.

Computation of the sonarbeam and source level

Using the position of the animal at click emission and the received signal at the 15 hydrophones, the sonarbeam one meter in front of the animals head was computed using cubic spline interpolation. The individual hydrophone sensitivities (+/- 1-2 dB) and the transmission loss between the animal and each hydrophone were accounted for. The sonarbeam was computed for the frequency range between 100 and 170 kHz, which contains the main energy of porpoise echolocation clicks (Villadsgaard et al., 2007). According to Møhl et al. (2000) the maximal intensity of the sonarbeam is termed the apparent source level (ASL) as the recording angle relative to the acoustic axis is unknown. Clicks for which the maximal intensity was directed at least one degree away from any array edge towards the center, were assumed to be recorded on the acoustic axis, hence resulting in the SL. In order to describe the beam and to compare with previous measurements, the beam is sliced into 12 radii each separated by 30° ranging from 0° (center of beam) to 20° off the acoustic axis (figure 1).

Subsequent analysis

In order to include only clicks that were directed towards the center of the array when estimating beam width, clicks were only considered when their maximal intensity was direct at least 1° away from any edge of the array. Since all three animals were scanning especially in the horizontal plane only a limited set of clicks out of every approach was directed at the center of the array, allowing to determine the -3 dB BW beam width for 305 out of the 1167 clicks.

IV. Results

Swimming behavior

Localizations of the emitted clicks were only possible at distances between 11.9 and 1.7 meter from the array. During the last part of the approach the animals turned parallel to the array and localizations of these clicks were not possible. Independent of their starting position, all three animals approached the prey directly without pronounced horizontal or vertical deviations. In some trails, the animals descended during the last four meters of the approach (figure 2, 3).

Inter Click Interval

The ICI of the emitted clicks decreased slightly from 30-40 ms to 20-30 ms as the animals approached the prey item. The ICI was always larger than the two-way-travel-time to the array and even at a distance of less than two meters from the array above 20 ms (figure 4, 5).

Click intensity

The received amplitude at one hydrophone varied by more than 12 dB within a few clicks (figure 6). Combining the data from all 15 array microphones and the position of the animal at click emission allowed to compute the (A)SL based on the sonar equation. The click intensity remained constant during the first part of the approach. At a distance of six to five meters from the array, two animals reduced the SL by 10 dB per halving of distance, one animal kept the SL constant (figure 4, 7,8).The maximal SLs of Eigil and Sif were similar, Freja emitted clicks that were 8-10 dB more intense.

Emission direction

While approaching the array directly, the emission direction was not always directed in the direction of body movement but showed variation, especially in the horizontal plane, causing strong oscillations in the RL of single receivers (figure 6, 9).

Directionality

The one sided -3 dB BW of 90 clicks emitted during a single approach of one animal showed strong variation (figure 10). Most of this observed variation is due to clicks directed at the edge or outside of the array making precise beam measurements impossible. The one sided -3 dB BW of 40 clicks that were directed at least one degree from any array edge during this single approach ranged between 4.5 and 7°, averaging at 5.83°. The average one sided -3 dB BW of all 74 clicks emitted towards the center of the array by this animal is 5.85° (figure 11). The beam width remained constant as the animal approached the prey item and array. The average one sided -3 dB BW of all three animals was 5.69° (n=305, figure 12). The one sided -3 dB BW of the second male porpoise Sif was 5.90° (n=111), the one sided -3 dB BW of the larger female Freja was 5.41° (n=120). The one-sided beam widths of Sif and Eigil did not differ significantly, however Freja had a significantly ($p<0.001$) narrower one-sided beam than both of the males (Mann-Whitney U Test with Holm-Bonferoni correction). Further off axis, the one sided -6 dB BW could be measured for 277 clicks emitted by all three animals and averaged 8.08°. The one-sided -6 dB BW of Eigil (8.18°,n=62) and Sif (8.31°, n=103) did not differ significantly, however Freja had a significantly ($p<0.001$) narrower one-sided beam (7.79°, n=112) than both of the males (Mann-Whitney U Test with Holm-Bonferoni correction) (figure 12,13,14).

V. Discussion

The ICI has been used to define different behavioral stages of foraging in bats and has recently been adopted for toothed whales (Verfuss et al., 2009). The constantly decreasing ICI from 30-40 ms to 20-30 ms matches the decrease of ICI in previous studies on the same animals nicely (Verfuss et al., 2009; Verfuss et al., 2005; Wisniewska et al., 2012) The ICI was always greater than the two-way-travel-time plus lag time, allowing processing the echo before emitting a new click. No clicks with an ICI lower than 20 ms were considered in the analysis as the animals turned parallel to the array during the last part of the approach.

Source level and variation thereof

The maximal SLs from 160 to 170 dB pp re 1 µPa at 1 m measured here matches previous ASL measurements of the same animals in captivity (Atem et al., 2009), however SLs of up to 205 dB pp re 1 µPa at 1 m of porpoises in the field have been reported (Kyhn et al., 2013; Villadsgaard et al., 2007), indicating that animals in captivity emit clicks of lower intensity (Villadsgaard et al., 2007).

The three individuals differ in SLs and variation thereof. Freja emitted the most intense clicks, the intensity of clicks emitted by Sif were 8 dB and clicks by Eigil 10 dB the less intense. Differences in click intensity between the three individuals had been reported earlier. The same differences in SLs were reported by Atem et al. (Atem et al., 2009) who found Freja emitted the most intense clicks and Eigil and Sif both clicks of comparable intensity, 7 dB lower than Freja. Using tags attached to two of the animals, Sif's clicks were found to have 5-10 dB higher amplitude than Eigil's clicks (DeRuiter et al., 2009).

Changes in received signal amplitude are either due to changes in the emission amplitude, changes in emission direction, or a combination of both. By recording an echolocator using a single receiver, statements on the emitted intensity are not possible. Recording echolocation signals with an array of receivers allows to measure the distance to the source and based on the sonar equation the transmission loss and hence the ASL. However the directional nature of echolocation signals does not allow to determine the SL unless the beam is sampled on the acoustic axis. Using a large planar array, RL variations at one receiver have been linked to either scanning movements, as observed in common pipistelle bats (Seibert et al., 2013) or to variations of the SL as reported for big brown bats (Koblitz et al., 2010). The variation of RL at one hydrophone in the present study can be explained by a combination of two effects. Two of the animals reduce their SL by 10 dB per halving of distance starting at a distance of six to five meters from the array in addition to scanning their beam over the array.

This target range dependent SL reduction has been reported for multiple toothed whale species, bottlenose dolphins (Jensen et al., 2009), harbor porpoises (Atem et al., 2009; DeRuiter et al., 2009) and other delphinids (Au and Benoit-Bird, 2003). In all previous studies the ASL reduction approximated 6 dB per halving of distance [20*log (distance)]. This range dependent SL reduction results in a constant SPL impinging on the target, the received echo still increases by 6 dB per halving of distance. However in addition to reductions in the outgoing signals, the auditory sensitivity in porpoises is reduced by 6 dB per halving of target range, resulting in a constant perceived echo amplitude (Linnenschmidt et al., 2012). Puzzling is that for two of the animals we find a SL reduction of 10 dB per halving of distance [33*log(distance)], resulting in a decrease of the perceived echo amplitude when combined with the sensitivity adjustments of the receiver. One of the animals does not show any range dependent SL reduction, indicating the need to investigate this further.

Beam width

The one sided -3 dB BW of Eigil approaching the large planar array capturing fish is 5.85°. The directionality of the same animal was under investigation in an earlier study (Koblitz et al., 2012). In this previous study, the same animal was trained to swim into a chin rest and voluntarily echolocate towards a plus shaped, narrow spaced hydrophone array. The average one sided -3 dB BW in this previous study was 5.93°, so within 0.1° of the present study. Using the same animal, but two different arrays, two different behavioral task and different analysis methods resulted in remarkably similar average beam widths. The small plus shaped array is an optimal setup when studying stationary animals directing its beam at the center hydrophone of the array. However, free ranging animals make it notorious difficult to record the narrow echolocation beam on the acoustical axis. Using the recordings made with the large 2D planar array with a hydrophone spacing of 0.5 m, we applied cubic spline interpolation to derive the direction of and the intensity emitted on the acoustic axis. The similarities of the two studies using the two methods indicate that the planar hydrophone array and associated processing methods do yield comparable results to the narrower spaced recording setup. The directionality of the clicks emitted by harbor porpoises had been measured twice, each study using a single animal (Au et al., 1999; Koblitz et al., 2012). Au et al. (1999) used a linear array in the vertical and horizontal plane separately and measured a -3 dB BW of 16°, the widest of all toothed whales investigated. Koblitz et al. (2012)

measured the directionality of another individual using a different, two-dimensional setup and found the beam to be more directional with an average -3dB BW of 12°. It remained unclear if this difference was caused by studying two individuals and hence resembles individual variation or if this difference was caused by the different methods. By doubling the sample size of available porpoises beam measurements to a total of four, we find that the ecological beam width is uniform among three individuals of this species (figure 13, 14). The -3 dB BW ranged between 10.8° and 11.8°, the -6 dB BW from 15.6° to 16.6°. Most pervious measurements on the emission directionality of toothed whales have been obtained from stationary animals with a linear array in the horizontal or vertical plane separately (Au et al., 2012; Au et al., 1978; Au et al., 1999; Au et al., 1986; Au et al., 1995; Au et al., 1987; Branstetter et al., 2012; Evans, 1973). Measuring the directionality of a beam that is not pointed directly at the array will result in an under determination of the directionality. The present study is one of two studies we are aware of that perform click by click based two-dimensional beam measurements of free toothed whales. This study confirms that porpoises in captivity emit a narrow sonar beam similar in directionality of other smaller toothed whales. Similar beam widths have been measured of harbor and Dall's porpoises in the field with a linear array (Kyhn et al., 2013). The narrow beam allows to focus the available energy forward and to increase detection distance on the acoustic axis given a certain amount of energy at disposal. In vespertilionid bats, signal frequency and emitter size have recently been found to be anti-correlated leading to a constant directionality independent of the animal's size (Jakobsen et al., 2013).The same concept could apply to smaller toothed whales where smaller species emit clicks of higher frequency content resulting in the same directionality of larger toothed whale species emitting clicks with lower frequencies.

Changes in beam width have been measured during the last phase of the approach, when the animal emits clicks at very small ICI during the buzz (Wisniewska et al., in prep). Since we only included clicks emitted with an ICI of 20 ms or greater in the analysis, we did not observe a change in beam width. We did not see any asymmetry of the beam as a previous study has shown (Koblitz et al., 2012), this could be due to the lower spatial resolution of the present planar array compared with the plus-shaped array.

Scanning

All three animals under investigation changed the emission direction relative to the swim path, i.e. scanned their environment with the sonar beam. Given the narrow sonar beam with a -3 dB BW of 10°, scanning can be used to counterbalance this narrow beam and still probe large volumes of water while foraging. In addition, scanning might aid in the spatial separation of targets by not only relying on directional hearing abilities but gather information on the angular position of an object by the direction of click emission. The main scanning observed in this study was seen in the horizontal plane. This could be due to the shallow holding pool allowing the animal to ensonify the whole vertical water column without scanning. As porpoises inhabit shallow waters, the authors hypothesize that the strong horizontal scanning component is not only due to the experimental setting but would be observed in wild porpoises as well. Future studies in the field needed to investigate how animals utilize the narrow beam there. Scanning in a target selection trial has been observed in both horizontal and vertical plane and was at least partially due to whole body movements, but also head movements independent from body movements (Wisniewska et al., 2012). The array used in this study did not have the aperture to cover the extent of the scanning completely. By using an array covering a larger area, the angular changes in emission direction could be studied to a greater extent.

SL and beam width and animal size

Three animals of known age and different weight were studied. Two animals, a 12 year old male and a 5 year old female weighed 42 and 48 kg respectively. The third animal, a 12 year old female was considerably heavier (59 kg) and bigger. The SLs of the emitted clicks differed between the three individuals, the heaviest emitting the most intense clicks, the two smaller individuals emitted clicks of comparable intensity. Not only did the SLs of the emitted clicks differ between the individuals but the directionality differed as well. The two smaller individuals emitted clicks with similar one-sided -3 dB BWs, the larger female emitted clicks of significantly higher directionality.

Directionality is determined by two physical properties: The size of the emitter and the wavelength, the latter being determined by the speed of sound in the medium and the emitted frequency. Toothed whales can theoretically only increase the directionality by increasing either the aperture or the frequency. As the frequency of porpoise clicks varies only slightly between individuals, different sizes in the aperture seem to be the only variable to achieve differences in directionality. The small but significant differences in observed beam width could indicate that the larger animal with a presumably larger transducer achieves a higher directionality. In addition, the SLs of the clicks emitted at distances greater than 4 m from the array seem to show a correlation to the beam width and hence animal size. The largest animal has a significantly narrower beam and higher SLs than the two smaller animals which do not differ significantly in neither aspect (figure 15). The higher SLs of the larger animal could be a byproduct by the higher directionality. By directing the click energy forward, the detection distance on the acoustic axis is increased while reducing the echo amplitude from objects in the periphery.

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VIII. Figures

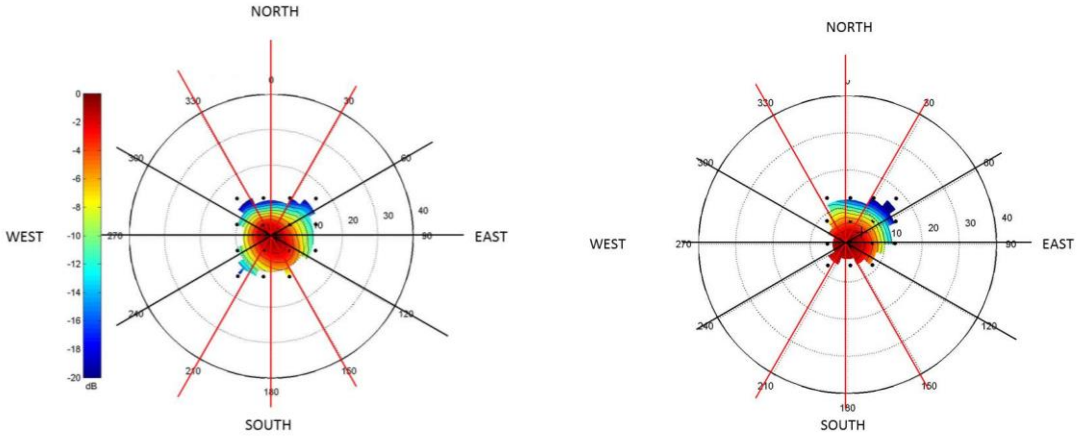

Figure 1: Polar representation of two emitted sonar beams. The black dots denote the 15 hydrophones. The center of the polar plot is the measured direction of maximal emission. The intensity is color-coded relative to the on-axis intensity.

Left beam: The -3 dB BW can be computed for all 12 radii separated by 30° each. The average one-sided -3 dB BW is computed by averaging over all 12 radii.

Right beam: The -3 dB BW cannot be computed for the lower (SOUTH) and left (WEST) radii as this beam was directed towards the lowed edge of the array. However the remaining part of the beam is nicely covered by the array and -3 dB BW can be measured. The average one-sided -3 dB BW is computed by the radii available.

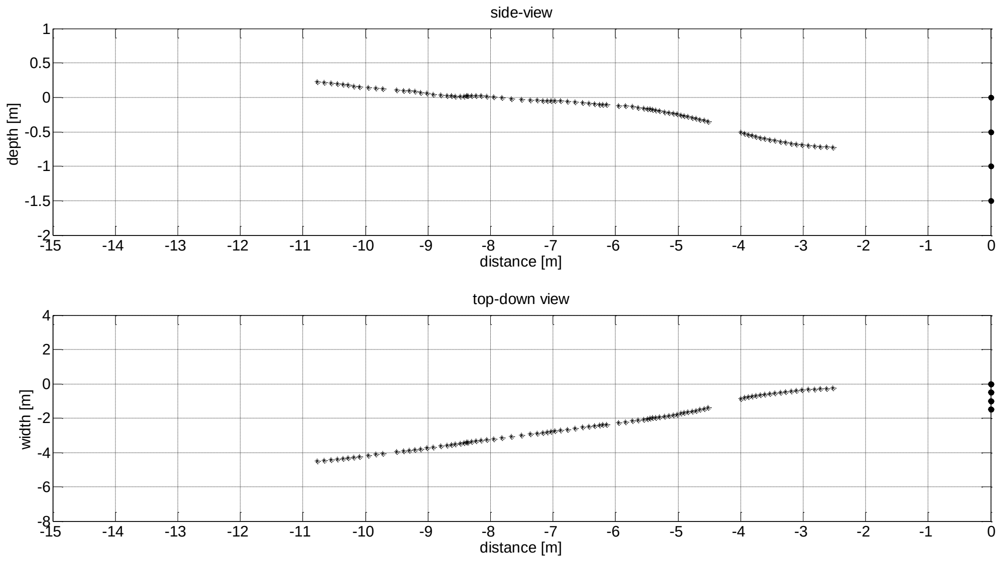

Figure 2: Position of localized echolocation clicks emitted by one animal during one trial (black stars) Receivers are shown as black dots. At a distance of 4.5 to 4 m, the animal was not directing its beam to the array. A: side view. B: top-down view with a different scale on the y-axis.

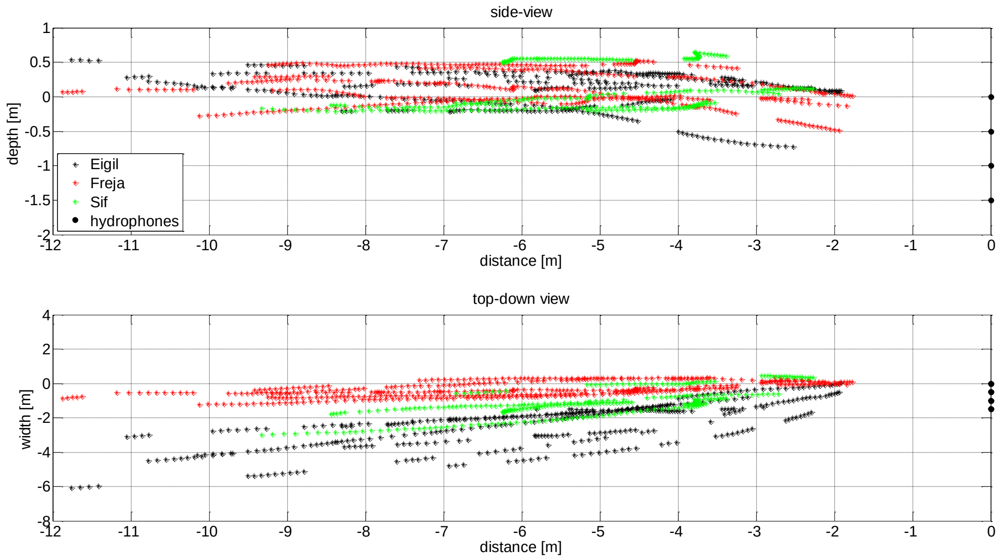

Figure 3: Position of all localized clicks (n=1167), different colors indicate the three animals.

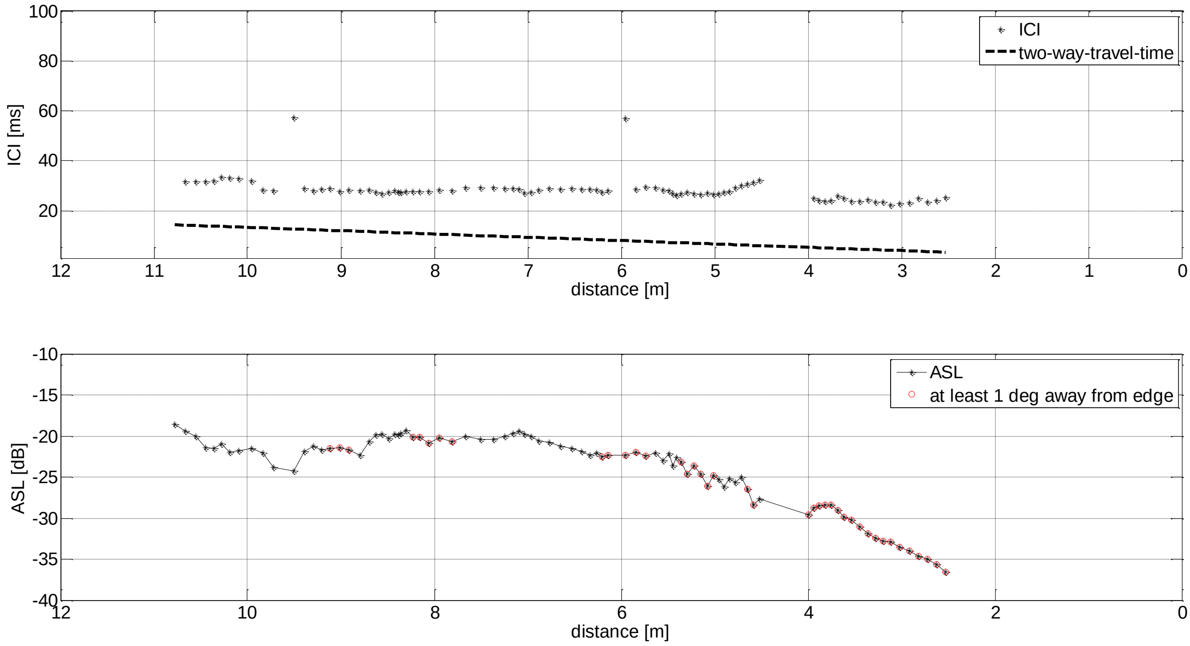

Figure 4: Top panel: Inter click interval of clicks emitted by one animal during one approach. The dashed line represents the two-way-travel time to the array. Lower panel: ASL of each click (black stars) and SL of clicks that were directed at least 1° from any edge of the array (red circles).

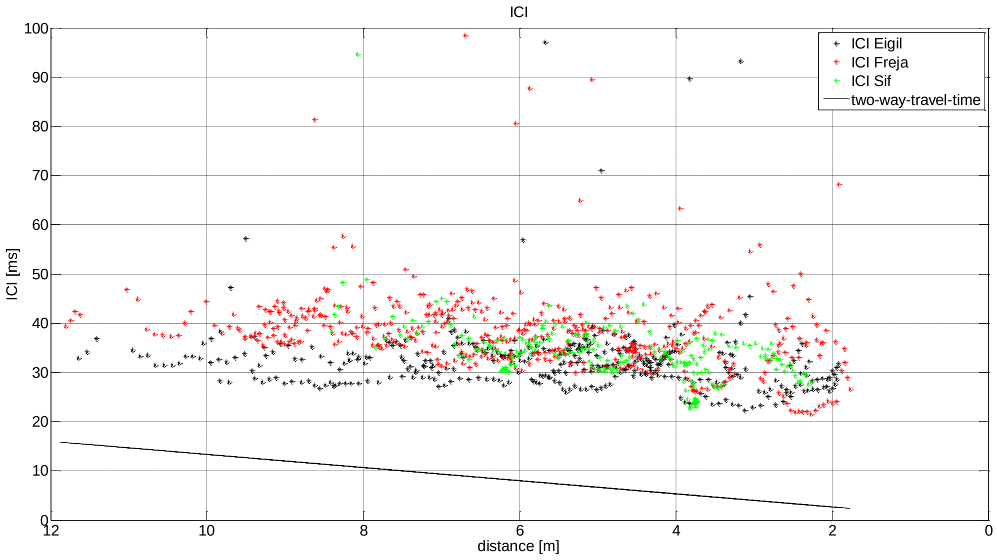

Figure 5: ICIs of all localized clicks (n=1167). All considered clicks were emitted with an ICI above 20 ms.

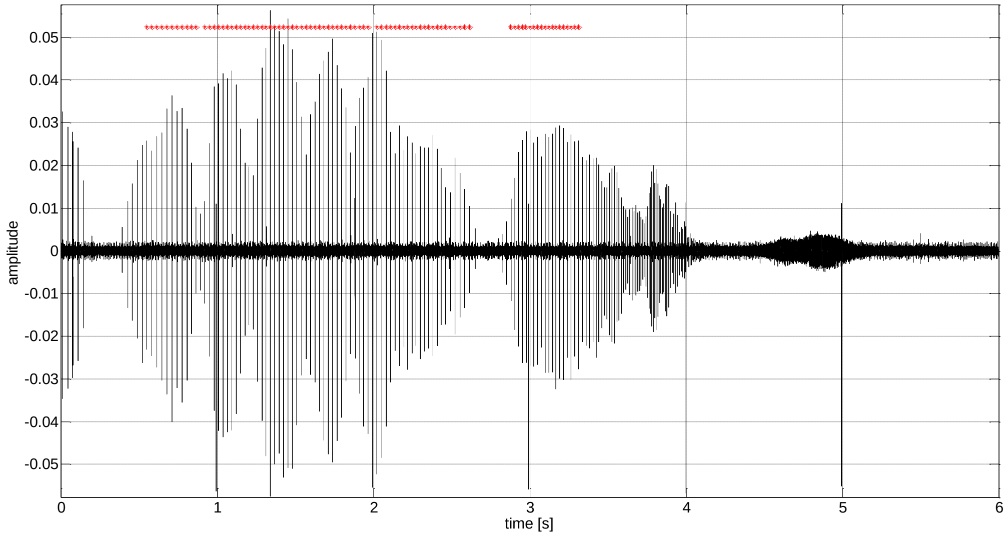

Figure 6: Typical echolocation sequence of a porpoise approaching the prey item. Each recording lasted 6 seconds (x-axis). The received click amplitude (y-axis) and the inter click interval vary. Clicks for which the position of the animal was determined are marked with red stars. Click-like signals occurring every second on the second are synchronization pulses.

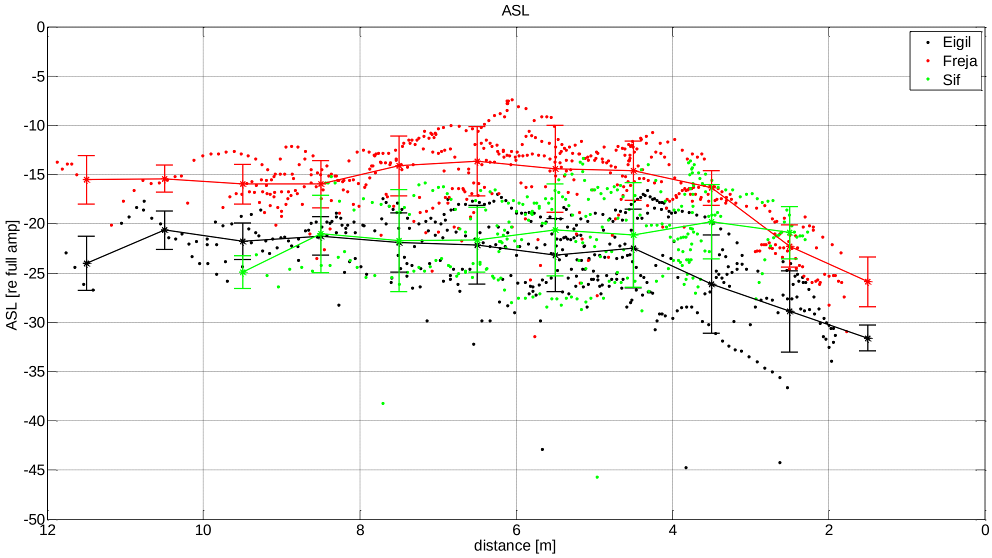

Figure 7: Mean ASLs and standard deviation per animal in one meter distance classes for the three animals (n=1167).

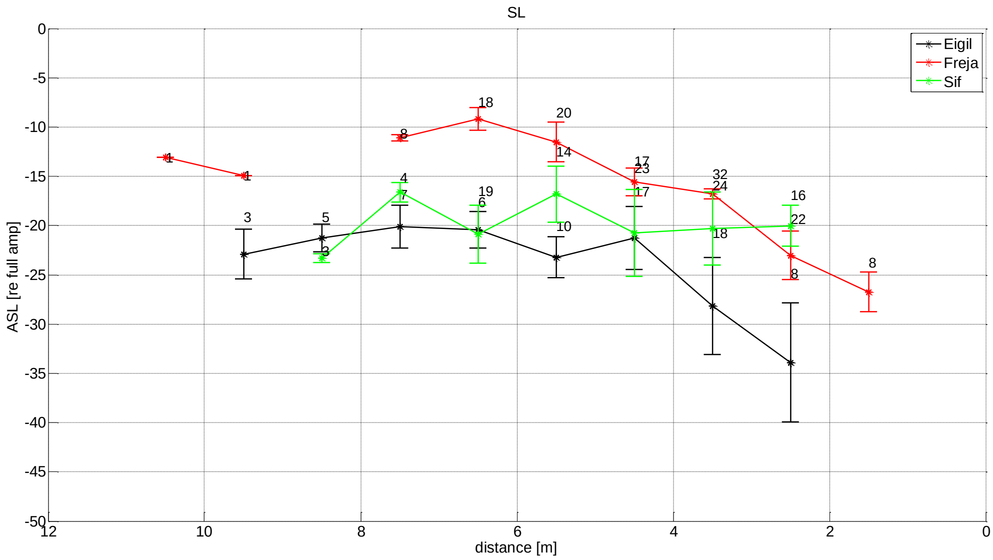

Figure 8: Mean SLs and standard deviation per animal in one meter distance classes. ASLs of clicks that were directed at least 1° towards the array center away from any edge of the array are assumed to provide real SLs. (n=305).

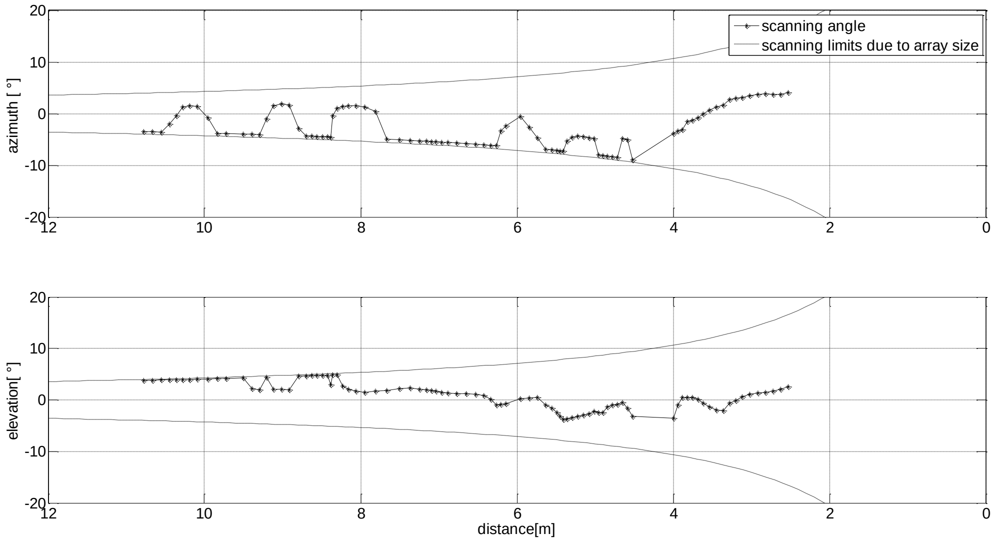

Figure 9: Changes in click emission direction of one animal during one approach. For every click, the angle between the direction to the center of the array and the (apparent) direction of click emission is plotted separately for the horizontal (azimuth) and vertical plane (elevation). The dashed line shows the angle between either edge of the array to its center as seen from the animals position and assuming it is centered in front of the array.

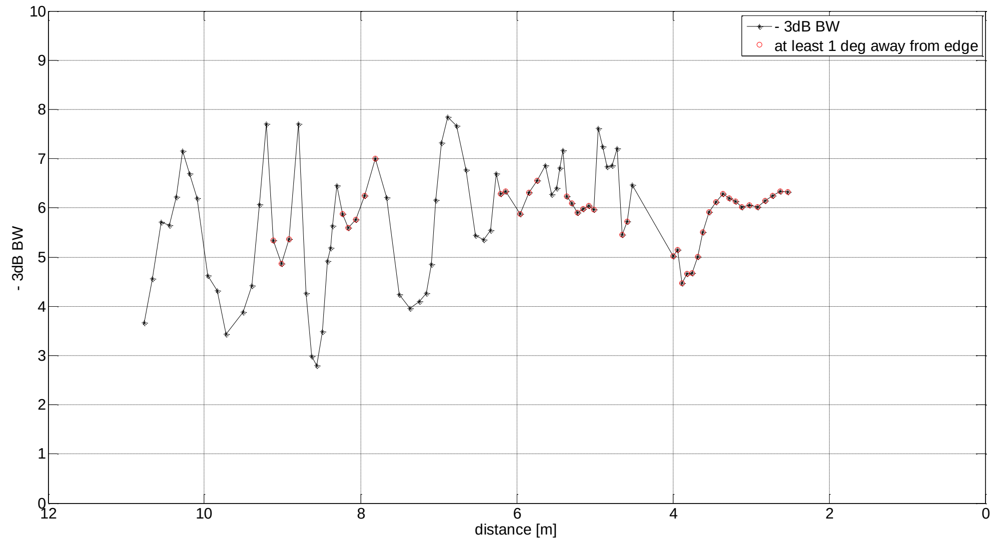

Figure 10: -3 dB BW of the 90 localized clicks by one animal during one approach (black stars), 40 of those directed at least 1° from any edge of the array (red circles).

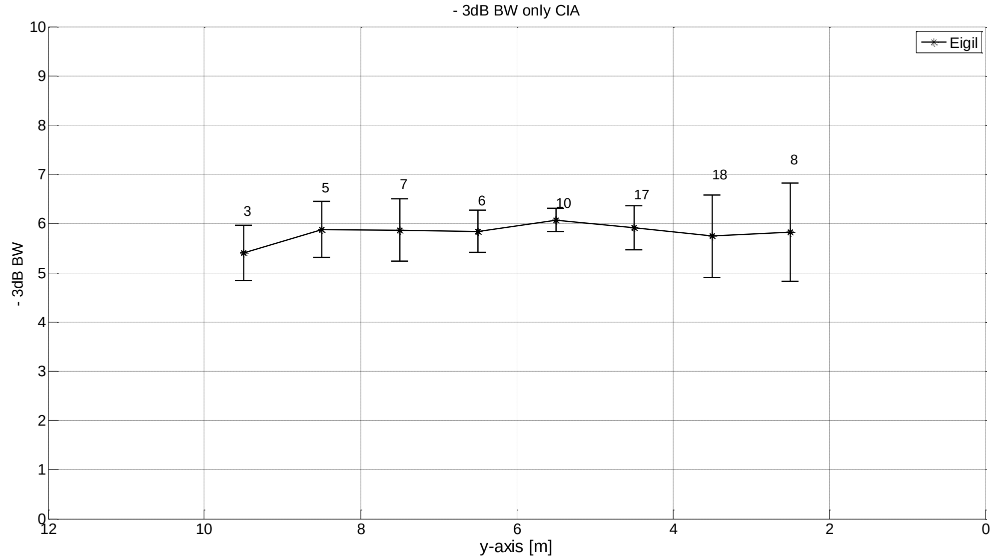

Figure 11: The -3 dB BW of one animal during seven prey captures. Clicks directed at least 1° away from array edge (n=74).

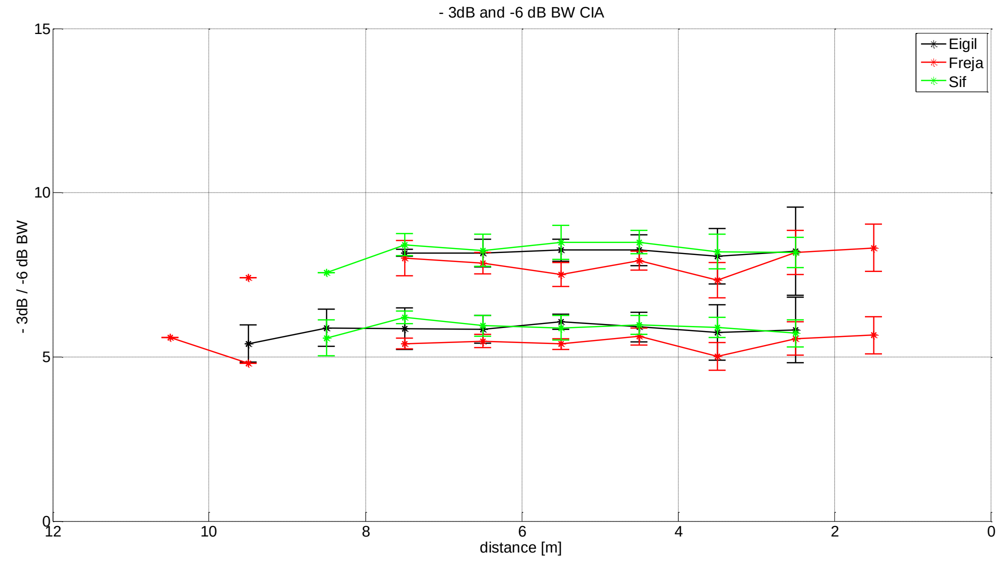

Figure 12: Both -3 and -6 dB BW separately for the three animals. Only clicks that are directed at least 1° away from edge of array are considered ($N_{-3\ dB}$=305; $N_{-6\ dB}$=277).

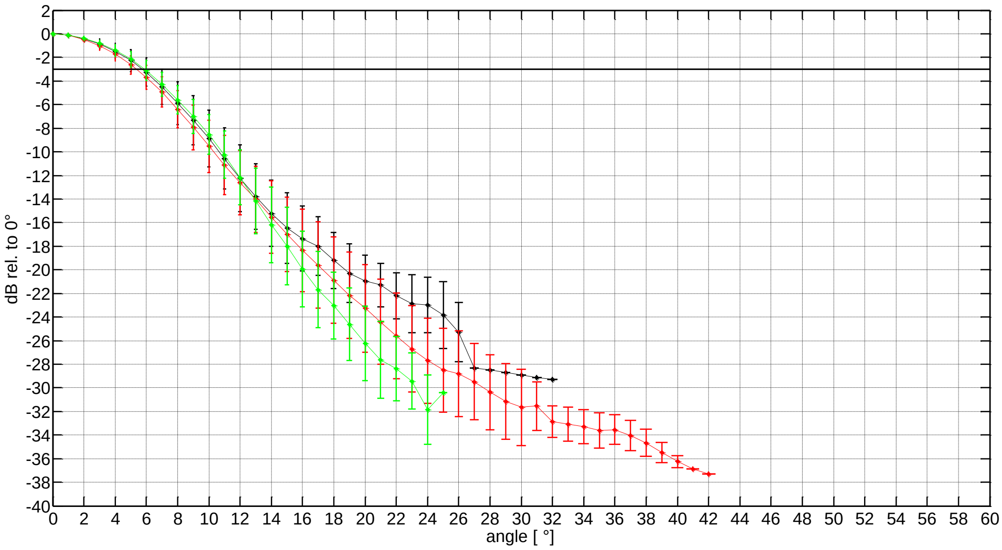

Figure 13**:** Composite beams for each animal. Rotational symmetry was assumed and all radii were averaged. Only few data points were available further than 20° off axis due to the small size of the array (Eigil: black, Freja; red, Sif; green).

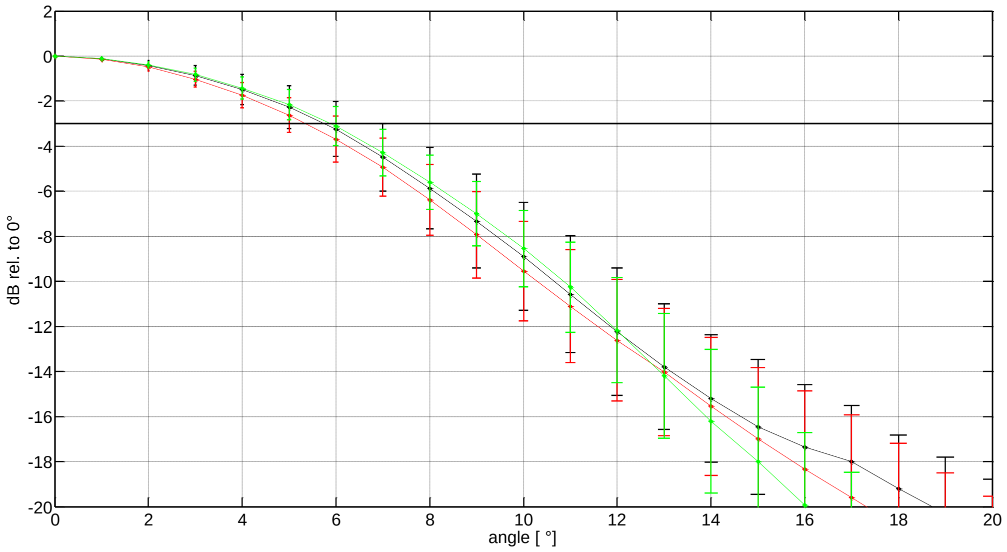

Figure 14: Beam pattern for the first 20° off axis, where the beam was sufficiently sampled (Eigil: black, Freja; red, Sif; green).

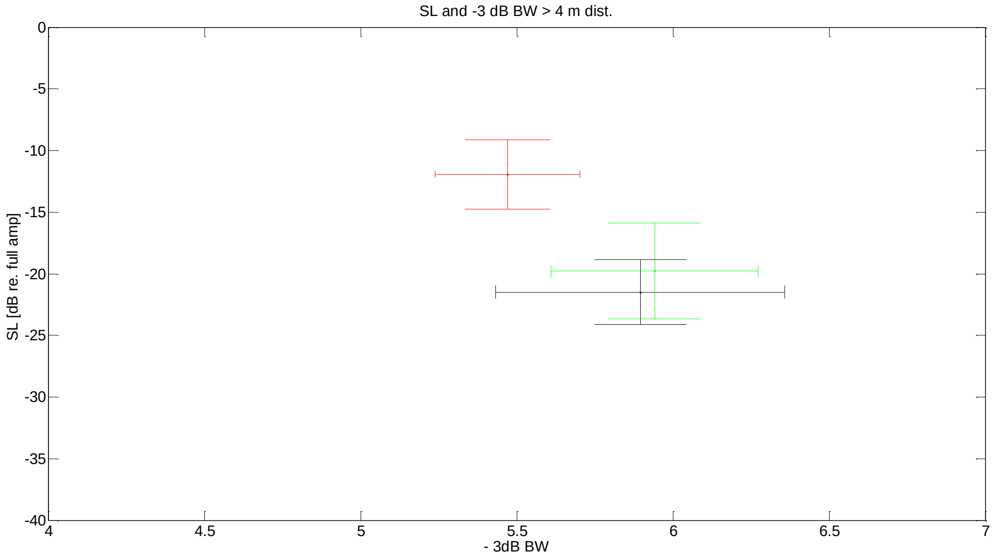

Figure 15: -3 dB beam width vs. SL for clicks emitted when the animals were further than 4 m from the array (Eigil: black, Freja; red, Sif; green).

6 Conclusion and Outlook

6.1 Summary

Since I started my doctorate, great scientific advances were made in researching the directionality and intensity of echolocation signals of bats and toothed whales.

6.1.1 Signal intensity

Detailed SL measurements in both taxa in the field show that intensity measurements of animals in captivity underestimated the SL used in the wild (Brinkløv et al., 2010; Jakobsen et al., 2013a; Surlykke and Kalko, 2008; Villadsgaard et al., 2007). Even bats termed “whispering” bats emit calls with 110 dB SPL rms re 20 µPa at 10 cm (Brinkløv et al., 2009), however “stealth echolocation” is used by barbastelle bats that hunt for eared prey (Goerlitz et al., 2010).

Array measurements and telemike recordings confirm that both taxa utilize target range dependent SL reduction (Atem et al., 2009; Au and Benoit-Bird, 2003; Hiryu et al., 2007; Jensen et al., 2009; Koblitz et al., 2011; Wisniewska et al., 2012). Most studies also confirm a target range dependent SL reduction of 20*log(distance) leading to constant SPL at the reflector(Atem et al., 2009; Au and Benoit-Bird, 2003; Hiryu et al., 2007; Jensen et al., 2009; Koblitz et al., 2011; Wisniewska et al., 2012). The increase of the echo at the animals receiving system, given this 20*log(distance) SL reduction, is compensated for by a range dependent sensitivity adjustment of the receiver of an additional 20*log(distance) (Hartley, 1992a; Linnenschmidt et al., 2012a).

This SL reduction is not constant but varies with wingbeat, high intense calls are emitted during the last part of the upstroke, calls with an in average 4 dB lower intensity are emitted during the downstroke (Koblitz et al., 2010).

6.1.2 Directionality

Multi receiver arrays have been used extensively over the past eight years to study the directionality of freely moving **bats and toothed whales**, either in captivity or in the field leading to a strong increase in the number of species investigated, allowing first comparative studies. Based on the relationship of emitter size, frequency and speed of sound, the directionality index (DI) in bats (DI of ~ 10-16 dB) is much smaller than in toothed whales [DI of ~ 24-32 dB, figure 20 , Madsen and Surlykke (2013)]. The frequencies utilizes by both taxa are in the same order of magnitude, but the transmitting aperture in bats is approximately one order of magnitude smaller than in toothed whales and the speed of sound in air is almost five times slower than in water (Madsen and Surlykke, 2013).

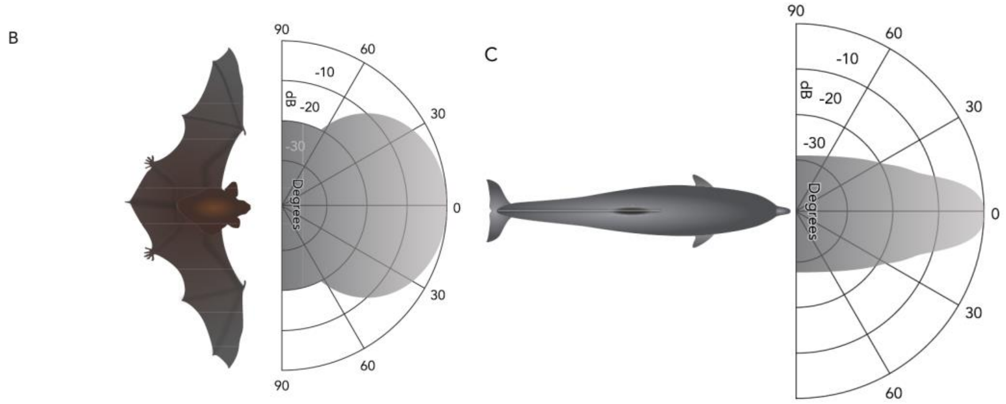

Figure 20 : Comparing the beam width of the two taxa [from Madsen and Surlykke (2013)].

The directionality of six vespertillionid **bat species** remained constant, despite a variation in forearm length from 32 to 54 mm which correlates to gape height (figure 21). This difference in emitter size is compensated by smaller bats emitting signals with higher frequencies, resulting in the same directionality that larger bats obtain with lower frequencies [Jakobsen et al., (2013d), figure21]

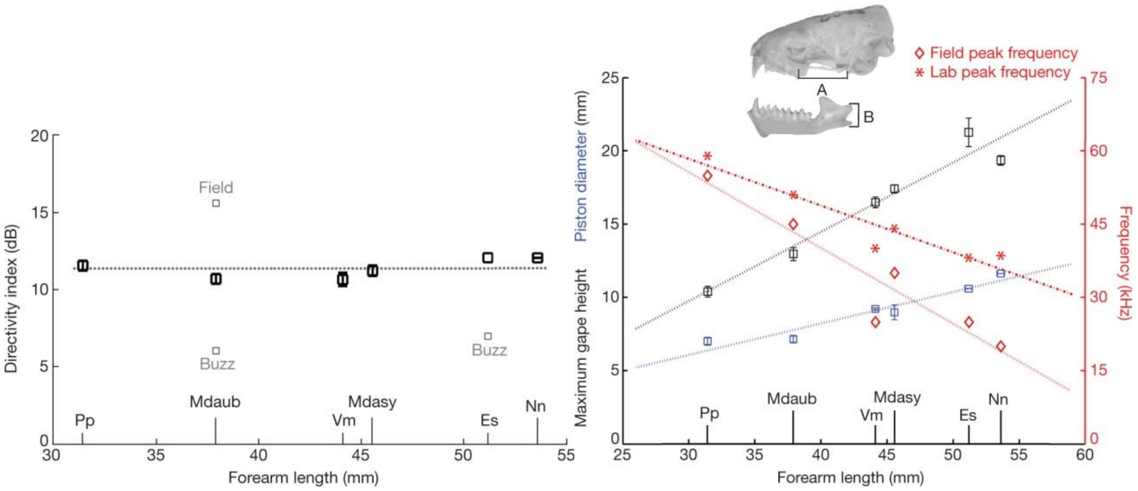

Figure 21: The same directionality is achieved independent of forearm length (left). The differences in emitter size are counterbalanced by call frequency [right figure, Jakobsen et al (2013d)]

Among **toothed whales** a dedicated comparative study under the same conditions has not been carried out yet. The pattern that larger odontocetes use lower frequencies is also observed in this taxa. If this leads to similar DIs among toothed whales or if this simply increases detection distance in the larger, low frequency emitting animals has yet to be investigated. Looking at all beam measurements available, no clear trend between size of the animal and directionality index is apparent (figure 22). The directionality of the harbor porpoise has to date been measured four times: Twice of stationary animals (Au et al., 1999; Koblitz et al., 2012), once of three freely ranging animals in captivity (Koblitz et al., in prep-d) and once of animals in the field (Kyhn et al., 2013). Three of those studies result in DIs of 24-25 dB (Koblitz et al., 2012; Koblitz et al., in prep-d; Kyhn et al., 2013), showing that the directionality of one of the smallest toothed whale echolocating at 130 kHz is comparable to the directionality of twice as large delphinids using centroid frequencies between 60 and 100 kHz (Wahlberg et al., 2011c). Recent measurements of the directionality of two other small NBHF species support this trend of small animals achieving a high directionality (Kyhn et al., 2010). Achieving high directionality might have been an evolutionary force acting upon small toothed whales, resulting in the evolution of high frequencies, despite the increased attenuation and thus reduced detection distance. Another hypothesis suggests that high frequency echolocation clicks in the NBHF group have evolved in an acoustic arms race between NBHF species and their predator, the killer whale (Morisaka and Connor, 2007). The highest directionality has been measured for beluga echolocation clicks (Au et al., 1987). This species has a large, pronounced melon that might cause this high directionality.

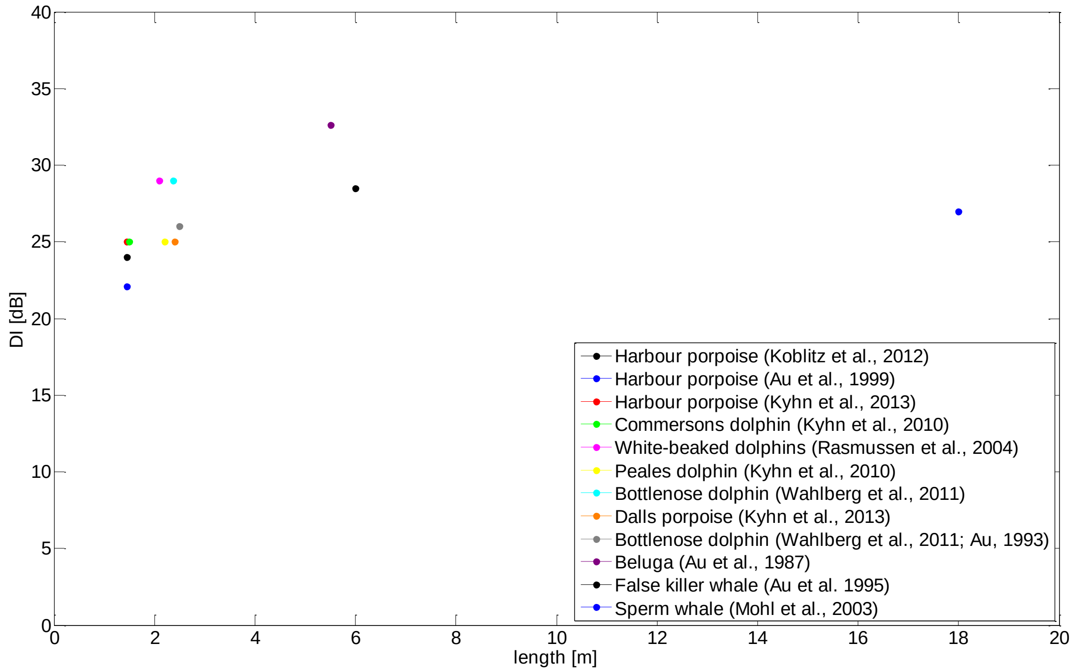

Figure 22: DI measurements of different tooth whale species in relation to animal length (when the species showed sexual dimorphism, the length of males was used).

Individual differences of the directionality within any bat species have to my knowledge not been studied. In toothed whales the author has been able to show that the ecologically important directionality between three individuals did not differ. However there is a small but significant difference in the -3 and -6 dB BW which correlates to animal weight. The largest individual emitted clicks with higher directionality (Koblitz et al., in prep-d)

Single individuals of both taxa adapt their directionality depending on the behavioral task they are faced with. Bats widen their beam during the last phase of a prey approach by either lowering the emission frequency (Jakobsen and Surlykke, 2010) or presumably changes in noseleaf structure (Matsuta et al., 2013). Porpoises which do not have as much control over the emission frequency change their directionality in the same context during the last phase of prey capture (Wisniewska et al., in prep). Since emission frequency remains quasi constant, another mechanism of changing directionality appears to be at play. The observed changes of DI are most likely caused by configuration changes (either size and/or shape) of the emission apparatus, among those the fatty melon and air sacs.

	Directionality of bats	**Directionality of toothed whales**
Between taxa	🟥 Madsen and Surlykke, 2013	
Within one taxa	🟩 Jakobsen et al., 2013	🟩 Koblitz et al., 2012
Between individuals	🟨 -	🟩 Koblitz et al., in prep.
Within one individual	🟥 Jakobsen and Surlykke, 2010	🟥 Wisniewska et al., in prep.

Table 2: Summary of differences in directionality (red), similarity in directionality (green) or data deficiency (yellow), between and within taxa as well as between and within individuals of one taxa.

6.2 Outlook on future array development and use

The use of arrays, both under water and in air is increasing. Fast computers and ADCs at reasonable cost per channel and with good SR (0.5-1 MHz/channel) allow the use of more receivers and hence either better spatial resolution, a wider coverage or even a combination of both. Thus these larger arrays allow a more detailed study of the directionality of stationary animals in captivity (Gaudette et al., 2014; Kloepper et al., 2012a; Kloepper et al., 2012c; Koblitz et al., 2012).

In addition to the traditional parameters ICI or PI, duration and frequency, source level and directionality of free ranging animals are now parameters under investigation by combining the dual use of arrays for first acoustic localization and then subsequently measurements of those sonar parameters (Koblitz et al., in prep-b; Koblitz et al., in prep-d; Wisniewska et al., in prep). The main advantage of small portable array recording equipment is flexible use in the field. It is now possible to obtain multi receiver recordings in harsh environments, such as pack ice (Koblitz et al., in prep-b).

More complex arrays setups are nowadays used to study odontocetes and have the potential to be used for studies on bats as well. Combining acoustic localizations obtained relative to a moving array system with the position of the array from GPS sensors and its orientation from compass/motion sensors allows to geo-reference the movements of echolocators and combine those with the positons of features in the environment [e.g. sidescan data showing underwater topography or the positons of trees in a forest obtained by laser scanners or theodolites (Holderied et al., 2006)]. This allows determining the reaction of animals in relation to the distance to various objects [e.g. vegetation, man-made structures, objects of interest (Holderied et al., 2006; Koblitz et al., in prep-a)].

In addition to confirming or refuting measurements of parameters previously studied of animals in captivity (Surlykke and Kalko, 2008; Villadsgaard et al., 2007; Wahlberg et al., 2011c), interactions of echolocating individuals (Götze et al., in prep) and echolocator and their prey (Corcoran et al., 2013; Goerlitz et al., 2010) can be studied in great detail.

Larger arrays, both in dimensions to cover the obviously extensive scanning, as well as in number of receivers to increase the resolution are being designed. Recent improvements in ADCs (16 channels on one ADC board, 1MHz/channel, simultaneous ADC, 16 or 32 bit), fast data transfer rate from ADC to computer via PXI-Express cards and especially fast data storage using large SSD allows to record many more channels than only a few years ago.

6.3 Future research

6.3.1 Changes in the emission direction of the sonar beam

A first study on odontocetes has shown that the direction of the echolocation beam can be changed while the head is fixed on a bit plate (Moore et al., 2008), however if animals use this ability in the wild remains unknown. It would be hydrodynamically beneficial for odontocetes to steer the echolocation beam independent of body or head movements. In bats this change of signal emission independent of heads movement has to date not been studied. Horseshoe bats would be prime candidates for studying this beam scanning independent of head movements for multiple reasons: The nose leaf shape can be changed and should influence beam shape and/or emission direction. In addition, this taxa perches hence makes recordings easier. Focusing a laser vibrometer on the nose leaf and recording it in sync with a microphone array placed in front of the animal will allow to compare horseshoe movements to scanning movements.

In free ranging toothed whales, larger species would be Krogh's animal to investigate head movement independent beam steering. By placing a dtag [acoustic recorder with accelerometer (Johnson and Tyack, 2003)] on the animal’s head, the signals produced by the animal and the head orientation can be measured. Recording the same animal with a large hydrophone array will enable to measure the scanning movements. Comparing the two datasets should show synchronized head and beam scanning movements or scanning movements without corresponding head movements, which would indicate that at least that particular species is able to steer its beam without moving its head.

6.3.2 Correlation of animal size to frequency and directionality

In vespertilionid bats, the directionality has been shown to be independent of animal size. Larger animals emit echolocation calls at lower frequency but have a larger gape size, i.e. a larger emitter. These two factors, call frequency and gape size even out and lead to calls of similar directionality independent of animal size (Jakobsen et al., 2013d). Fewer directionality measurements have been done on free ranging toothed whales using the same array system or behavioral context. The sperm whale with its exceptional biosonar system will likely be an outlier and have a higher directionality than other groups of odontocetes. Directionality measurements of smaller odontocetes vary without showing a clear relationship to animal size or weight. The beam width of the largest of all delphinids, the killer whale, has not been measured yet and would be an ideal data set to obtain.

6.3.3 Ontogenetic changes of echolocation

Do echolocation parameters, such as SL, DI and frequency composition change between birth and maturity? This question would be best answered by studying individual animals in captivity and recording right after birth and then in regular intervals until they have reached maturity.

6.3.4 Mechanism of directionality changes in toothed whales

Jakobsen and Surlykke (2010) have shown that bats can change their directionality by frequency changes. Recently Wisniewska et al. (in prep) have shown that porpoises also widen their echolocation beam as they approach prey. The frequency content of the emitted clicks does not seem to cause these changes of directionality and it is assumed that melon and air sacs play a fundamental role. The mechanisms and structural changes that lead to changes in directionality remain to be resolved. The observed changes in directionality could be continuous or bimodal, with a high directionality in regular clicks and low directionality in buzzes.

6.3.5 Comparative studies of the sonar properties of both taxa

The initial goal of this thesis was to compare the directionality of porpoises and bats and to investigate differences in the use of the sonarbeam, e.g. differences in scanning behavior during foraging. However the development of the recording systems and describing the sonarbeam characteristics for each taxa did not leave time for comparative studies. I sincerely hope that these will be carried out in the near future.

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I had the great opportunity to participate in the summer course "acoustic communication" offered by the University of Southern Denmark. During this amazing course I met great fellow students some of whom became dear friends. Thanks for being there along the way, Lasse Jakobsen, Signe Brinklov, Alison Stimpert, Kristin Übernickel, Inga Geipel, Sophie von Merten, Frants Jensen, Holger Görlitz and Ben Falk.

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A great group of friends made the multiple stays and visits in Odense and Kerteminde unforgettable, thanks so much, Anne Grimsbjerg Pedersen, Anders Ellegaard, Jacob Lindgaard Nielsen, Kurt, Fliese og Krake, Lone Nørgaard Bruun, Vibeke Hepworth, Lara Delgado Garcia, Sanja Heikkilä, Caroline og Coralie.

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Peter Madsen, you are a great teacher, I now teach your dB class to my students and bug them with “What is that in dB?” Thank you for a great calibration session in Aarhus and your valuable input into this work!

Kim Lundgreen, you are the soul that keeps Marine Biological Research Centre going, lab manager and mom at the same time.

Friends far and near, you have been amazing over the years. Supportive and forgiving when I did not reply to your e-mails or return your calls. Thanks for being the best friends I could ask for, Julia Schmitz-Dräger, Caro Höschle, Caro Diziol, Alex Stober, Chantal Huijbers, Michelle Weirathmueller, Stefanie Rohrer, Timo Erdtmann and Lars Großmann.

Rowing was a great balance to all desk related work, I especially like to thank the Lehrteam Rudern at the University of Tübingen, the Homophile Duschgruppe and the Stralsunder Ruderclub for great workouts, regattas, coaching and social sessions.

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Friends and colleagues at German Oceanographic Museum for four great years in Stralsund, especially Kati Brundiers, Harald Benke, Stefan Bräger, Timo Moritz, Karin T. Clausen, Anja Gallus, Anne Hermann, Nadine Pankow, Annika Weseloh and Ria Schmechel. Julia Müller did a great job in compiling the sizes of toothed whales used in figure 22.

The amazing SAMABH crew: This project is amazing, despite the sweat, blood and tears of the past years. Thanks a lot for everything, Julia Calström, Ida Carlen, Cinthia T. Ljungqvist, Daniel Wennerberg, Len Thomas, Mats Amundin, Nick Tregenza, Louise Burt, Olli Loisa and Jamie MacAulay.

Along the long way I was fortunate to have professors and PIs who took interest in the progress of my PhD or, quite often, lack thereof, thanks to all of you. Two stood out especially, you have been mentors to me for about a decade, thank you so much, Jo Ostwald and Beren Robinson!

I would especially like to thank and remember three dear professors who have sadly passed away during the past years: Eli Kalko, Björn Siemers and Volker Böder. You all were a source of inspiration, amazing positive attitude and great teaches and collaborators. I miss you!

My great Canadian family and dear friends, Matt, Annette, Jim and Michelle McCoubrey, Eleanor Hopkins and Zorianna Zurba: It is great to have a second home away from home!

Last but most important, my family! Thank you for suffering and cheering with me, being there for me and kicking my rear when appropriate! Angelika, Achim, Thorsten, I cannot tell how much you mean to me and I am so grateful for all you have done for me! Enrico, Suzy and Ulrike, I am so happy you became family, thank you for your encouragement and especially Ulrike for two weeks in July when you watched Paula!

Laura, I am so sorry for the stress I put on you during the final stages of this doctorate, I will try to make up for it. I love you!

As I am finishing this at 2 am after a couple of Ouzos (thanks to the Kaloyiannis family), I am certain I forgot to thank great friends, colleagues and supporters along the way. If that happens to be you, please step forward and speak up, I owe you a beer.

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Always remember: DFTBA!

9 Curriculum Vitae

Dipl.-Biol. Jens C. Koblitz

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18439 Stralsund, Germany
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Education

University of Tübingen, Germany Sept. 2006-present

- Ph. D. Thesis: Studying the biosonar of bats and porpoises using multi receiver arrays

University of Tübingen, Germany Dec. 2005-July 2006

- Diploma Thesis: Sonar beam characteristics and scanning behaviour of the big brown bat (*Eptesicus fuscus*) during landing

University of Konstanz, Germany Oct. 2001-July 2006

- Major: Biology

University of Guelph, Canada Sept. 2003-Apr. 2004

- Academic exchange, Major: Marine and freshwater biology

Research and work experience

Research Associate Jan.2011-present
German Oceanographic Museum, Stralsund, Germany

- Coordinated multiple research projects on harbor porpoises (SAMBAH, COSAMM)
- Designed and used a multi-receiver array to record toothed whales in the wild
- Combined basic research on biosonar and static acoustic monitoring

Graduate Research Assistant Sept. 2006-Dec. 2010
Animal Physiology, University of Tübingen, Germany

- Developed and calibrated acoustic recording equipment for recordings in air and underwater
- Recorded bats and porpoises using a 16 receiver array
- Programmed Matlab algorithms to position animals and derive sonar beam characteristics

Intern Nov. 2006, June-Aug. 2004
Cascadia Research Collective, Washington, USA

- Designed and programmed a “computer-assisted-matching-database” for humpback whales
- Collaborated on line-transect and small-boat surveys, including marine mammal identification, taking photo ID’s and data recording
- Assisted in taking biopsies and deploying acoustic- and TDR-tags

Diploma Student Dec. 2005-July 2006

Department of Animal Physiology, University of Tübingen, Germany

- Trained bats for experiments in echolocation
- Synchronized video and ultrasound recordings, using a 16-microphone array and a stereo infra-red camera system
- Programmed, modified and used MATLAB routines for digital signal and 3D movement analysis

Research Assistant May-July 2005

Department of Biology, University of Konstanz, Germany

- Extracted DNA from ancient and fresh fish tissue samples
- Performed PCR, purified and sequenced nuclear- and mtDNA
- Processed data using software for multiple sequence alignment and phylogenetic analyses

Research Assistant Sept. 2003-May 2004

University of Guelph, Canada

- Successfully developed and ran experiments on anti-predator behaviour in fish
- Conducted boat electrofishing for sample collection
- Designed lab setup and executed detailed behaviour recording

Other work and volunteer experience

Canoe Guide

Sweden and Germany Aug. 2001-present

- Independently guided ecologically-focused canoe tours in Sweden and Germany with 24 participants of all ages
- Gained valuable experience in conflict management, leadership, communication, problem solving and decision-making

Rowing Coach

Düsseldorf, Konstanz, Tübingen, Stralsund, Germany June 1999-present

- Coached children and adults between the ages of 10 and 40
- Supervised daily work-outs and kept rowers focused and motivated
- Safely transported rowers and equipment to competitions, including boat loading, driving trucks and pulling trailers

Other achievements and interests

- Fluent in English and German, basic Danish, Spanish and French
- Standard First Aid certified, lifeguard trained, motor boat license
- Excellent computer and programming skills in: MATLAB, SPSS, MS Office
- Interests: competitive rowing for 19 years, marathon running, camping, photography and darkroom work, sailing, saxophone

Society memberships

- Society for Marine Mammalogy, European Cetacean Society, Acoustical Society of America

Conferences and workshops organized

- Workshop on “Calibration of Click detectors”, German Oceanographic Museum, Stralsund, 2011
- Workshop on “Acoustic Localization of Echolocating Mammals”, Kerteminde, Denmark, 2009
- Lakeshores 2003 Conference, Konstanz, Germany, 2003

Field experience (6 field trips, 16 weeks, between 18° N and 70° N)

- Bats: Mexico (3 weeks, 2008), Switzerland (1 week, 2008)
- Toothed whales: Denmark (PI, 4 weeks, 2013), Greenland (3 weeks, 2013), Wales (Co-PI, 3 weeks, 2012), Hawaii (2 weeks, 2006)

Teaching experience

- Workshop on Bioacoustics, German Oceanographic Museum, 2011 and 2012
- Introduction to Bioacoustics, FTZ Westküste, Büsum, 2011
- International PhD Course on Acoustic communication, Graduate School SNAK, Denmark, 2009
    - Lecture on the Identification of bats in the field
    - Practical on the directionality of loudspeakers
    - Bat excursion
- Graduate courses, University of Tübingen, 2007-2009
    - Student research projects in Animal Physiology
    - Bioacoustic field course with student research projects
- Workshops on marine mammals. “Dolphin Summer Camp”, Inverness, Scotland, 2005
- Teaching Assistant for a graduate course on Microbial Evolution, University of Konstanz, 2005

Co-supervised student theses

- Götze, S. MSc thesis. 2012. Interindividual interactions of hunting pipistrelle bats (*Pipistrellus pipistrellus)* [in German]
- Schaffeld, T. BSc thesis. 2011. Investigating the foraging behavior of porpoises (*Phocoena phocoena*) in selected areas of the Baltic Sea using static passive acoustic monitoring [in German]

Scholarships, travel grants and awards

- Travel grants from the Reinhold-and-Maria-Teufel-Foundation to attend conferences in Prague,Kyoto, Paris, Egmond aan Zee, 2008-2010
- Travel grant from the Graduate School on Sense organs, Nerve systems, Behaviour and Communication (SNAK) for a research visit at Fjord&Bælt, Kerteminde, Denmark, Oct. 2008
- Travel grant from the Reinhold-and-Maria-Teufel-Foundation for a research visit at Fjord&Bælt, Kerteminde, Denmark, Dec. 2007 and Sept. 2008
- Best Student Talk, European Cetacean Society Conference, Egmond aan Zee, Netherlands, 2008
- Travel grant from the Reinhold-and-Maria-Teufel-Foundation to attend PhD course “Acoustic Communication”, Denmark, 2006
- Scholarship from the “Landesstiftung Baden-Württemberg” to study at the University of Guelph, Canada, Sept. 2003-Apr. 2004

Peer-reviewed publications [3 first author, 3 co-author]

- Benke, H., S. Bräger, M. Dähne, A. Gallus, S. Hansen, C.G. Honnef, M. Jabbusch, J.C. Koblitz, K. Krügel, A. Liebschner, I. Narberhaus, U.K. Verfuß. 2014. **Baltic Sea harbour porpoise populations: Status and conservation needs derived from recent survey results.** Marine Ecology Progress Series. 495, 275-290

- Seibert, A., J.C. Koblitz, A. Denzinger, H.-U. Schnitzler. 2013. **Scanning behavior in echolocating common pipistrelle bats (*Pipistrellus pipistrellus*).** PLoS ONE 8(4): e60752. doi:10.1371/journal.pone.0060752

- Koblitz, J.C., M. Wahlberg, P. Stilz, P.T. Madsen, K. Beedholm, H.-U. Schnitzler. 2012. **Asymmetry and dynamics of a narrow sonar beam in an echolocating harbor porpoise**. Journal of the Acoustical Society of America. 131 (3), 2315-2324

- Koblitz, J.C., P. Stilz, W. Pflästerer, M. Melcón, H.-U. Schnitzler. 2011. **Source level reduction and sonar beam aiming in landing big brown bats (*Eptesicus fuscus*)**. Journal of the Acoustical Society of America. 130 (5), 3090-3099

- Koblitz, J.C., P. Stilz, H.-U. Schnitzler. 2010. **Source levels of echolocation signals vary in correlation with wing beat cycle in landing Big Brown bats (*Eptesicus fuscus*)**. Journal of Experimental Biology. 213, 3263-3268

- Robinson, B.W., A.J. Januszkiewicz, J.C. Koblitz. 2008. **Survival benefits and divergence of predator-induced behavior between pumpkinseed sunfish ecomorphs.** Behavioral Ecology. 19 (2), 263-271

Reports and non-peer reviewed publications

- Koblitz, J.C., K. T. Clausen, A. Gallus, S. Hansen, K. Brundiers, M. Kost, H. Benke. 2013. **SAMBAH Final report. The German contribution of harbor porpoise registrations in the Baltic Sea east of the Darss Sill as part of the SAMBAH-Project.** Final report to the Federal Agency for Nature Conservation

- Koblitz, J.C. 2012. **The Baltic Sea Harbour Porpoise (Phocoena phocoena): What do we know and what do we need to know?** In: Progress in Marine Conservation in Europe 2012: Proceedings of the Symposium Stralsund, Germany 18 - 22 June 2012. Eds.: von Nordheim, H., K. Maschner, K. Wollny-Goerke. BfN-Skripten 339. Federal Agency for Nature Conservation, Bonn, Germany, pp. 343-348. ISBN 978-3-89624-074-3

Invited presentations

- APL, University of Washington, Seattle, USA, May 2014
- STUKplus Conference, Berlin, Germany, October 2013
- 16th Annual meeting of the German Audiology Society, Rostock, Germany, February 2013
- Kiel University, Kiel, Germany, November 2012
- 3rd International Conference on Progress in Marine Conservation in Europe, Stralsund, Germany June 2012
- Ludwigs Maximilian University Munich, Munich, Germany June 2012
- MPI for Ornithology Seewiesen, Seewiesen, Germany, June 2012
- University of Windsor, Windsor, Canada, December 2006

Talks at conferences [8 as presenting author, 12 as co-author]

- Koblitz, J.C., K. Brundiers, M. Kost, L. Burt, L. Thomas, J. MacAulay, C.T. Ljungqvist, L. Mikkelsen, P. Stilz, H. Benke. 2014. **Determining the detection function of passive acoustic data loggers for porpoises using a large hydrophone array.** 167th Meeting of the Acoustical Society of America. Providence, USA

- Brundiers, K., M. Kost, L. Burt, L. Thomas, J. MacAulay, C.T. Ljungqvist, L. Mikkelsen, P. Stilz, H. Benke, J.C. Koblitz. 2014. **Detection functions of C-PODs: Determining the probability of detecting harbor porpoises using a large hydrophone array.** 28th Annual Conference of the European Cetacean Society. Liege, Belgium

- Carlström, J., L. Thomas, M. Amundin, J. Teilmann, J.C. Koblitz, N. Tregenza, I. Carlén, D. Wennerberg, L. Kyhn, S. Svegaard, , R. Koza, M. Kosecka, I. Pawliczka, C.T. Ljungqvist, K. Brundiers, L. Mikkelsen, J. Tougaard, O. Loisa, A. Galatius, I. Jüssi, H. Benke. 2014. **Large-scale static acoustic survey of a low-density population – estimating the abundance of the Baltic Sea harbour porpoise.** 28th Annual Conference of the European Cetacean Society. Liege, Belgium

- Carlström, J., L. Thomas, M. Amundin, J. Teilmann, J.C. Koblitz, N. Tregenza, I. Carlén, L. Kyhn, S. Svegaard, D. Wennerberg, R. Koza, M. Kosecka, I. Pawliczka, C.T. Ljungqvist, L. Mikkelsen, J. Tougaard, A. Galatius, O. Loisa, I. Jüssi, H. Benke. 2013. **Large-scale static acoustic survey of a low-density population – estimating the abundance of the Baltic Sea harbour porpoise.** 20th Biennial Conference on the Biology of Marine Mammals. Dunedin, New Zealand

- Sveegaard, S., J. Teilmann, A. Galatius, L.A. Kyhn, R. Dietz, J.C. Koblitz, M. Amundin. 2013. **Integrating genetics, morphology, acoustics and satellite telemetry in population management of harbour porpoises.** 20th Biennial Conference on the Biology of Marine Mammals. Dunedin, New Zealand

- Gonzalez-Terrazas, T.P., R. Simon, J.C. Koblitz, M. Tschapka, T. Fleming, R. Medellín, H.-U. Schnitzler, E.K.V. Kalko. 2013. **What Makes a Flower a Flower: The Bat Perspective.** 16th International Bat Research Conference. San Jose, Costa Rica

- Götze, S. J.C. Koblitz, H.-U. Schnitzler, A. Denzinger. 2013. **3D-Flight path reconstruction and echolocation behavior in *Pipistrellus pipistrellus* flying together do not indicate jamming avoidance**. 16th International Bat Research Conference. San Jose, Costa Rica

- Seibert, A.-M., J.C. Koblitz, A. Denzinger, H.-U. Schnitzler. 2013. **Using a 16 Microphone Array to Study the Scanning Behavior of *Pipistrellus pipistrellus* in the Field.** 16th International Bat Research Conference. San Jose, Costa Rica

- Nuuttila, H. K. Brundiers, L. Thomas, W. Courtene-Jones, P.G.H. Evans, J.R. Turner, J.D. Bennell, H. Benke, J.G. Hiddink, J.C. Koblitz. 2013. **Playback experiments to estimate the detection range of a click logger.** 27th Annual Conference of the European Cetacean Society. Setubal, Portugal

- Bräger, S., H. Benke, M. Dähne, A. Gallus, S. Hansen, C.G.Honnef, J.C. Koblitz, K. Krügel, A. Liebschner, I. Narberhaus, U.K.Verfuß. 2013. **A decade of acoustic monitoring in the Baltic Sea: Status and area use of two populations of harbour porpoises.** 27th Annual Conference of the European Cetacean Society. Setubal, Portugal

- Koblitz, J.C., M. Wahlberg, P. Stilz, P. T. Madsen, H.-U. Schnitzler. 2012. **The sonar beam characteristics and scanning behaviour of harbour porpoises during prey capture.** 26th Annual Conference of the European Cetacean Society. Galway, Ireland

- Koblitz, J.C., P. Stilz, H.-U Schnitzler. 2010. **Source levels of echolocation signals vary in correlation with wing beat cycle in landing Big Brown bats (*Eptesicus fuscus*).** 15th International Bat Research Conference. Prague, Czech Republic

- Seibert, A.-M., J.C. Koblitz, H.-U. Schnitzler. 2010. **The scanning behaviour of free ranging Barbastelle bats (*Barbastella barbastellus*).** 15th International Bat Research Conference. Prague, Czech Republic

- Gonzalez-Terrazas, T.P., J.C. Koblitz, M. Tschapka, T. Fleming, R. Medellín, H.-U. Schnitzler, E.K.V. Kalko. 2010. **Echolocation behaviour of the nectar-feeding bat *Leptonycteris yerbabuenae* approaching natural and modified flowers.** 15th International Bat Research Conference. Prague, Czech Republic

- Koblitz, J.C., P. Stilz, W. Pflästerer, M. Wahlberg, P.T. Madsen, A.-M. Seibert, H.-U. Schnitzler. 2009. **Studying the source levels, beam characteristics and scanning behaviour of free ranging bats and toothed whales using a two dimensional array of 16 receivers.** Workshop at the 5th Animal Sonar Symposium. Kyoto, Japan

- Koblitz, J.C., M. Wahlberg, P.T. Madsen, P. Stilz, H.-U. Schnitzler. 2008. **Two dimensional sonar beam characteristics of single harbour porpoise (*Phocoena phocoena*) echolocation clicks measured with a 16 hydrophone array.** Acoustics' 08. Paris, France

- Koblitz, J.C., M. Wahlberg, P.T. Madsen, P. Stilz, and H.-U. Schnitzler. 2008. **Directionality of individual harbour porpoise echolocation clicks.** 22nd Annual Conference of the European Cetacean Society. Egmond aan Zee, Netherlands

- Koblitz, J.C., P. Stilz, W. Plästerer, M. Melcón, H.-U. Schnitzler. 2006. **The sonar beam of big brown bats (*Eptesicus fuscus*) during landing.** 4th Joint Meeting of the Acoustical Society of America and the Acoustical Society of Japan. Honolulu, USA

- Robinson, B.W., K.J. Parsons, A.J. Januszkiewicz, J.C. Koblitz. 2006. **Developmental plasticity and divergence in sunfish.** 45th Annual Meeting of the Canadian Society of Zoologists. Edmonton, Canada

- Koblitz, J.C. and B.W. Robinson. 2004. **Variation in the behaviour of pumpkinseed sunfish (*Lepomis gibbosus*) ecomorphs under the risk of predation.** Ontario Ecology & Ethology Colloquium. Toronto, Canada

Posters at conferences [1 as first author, 15 as co-author]

- Kosarev, V., H. Benke, S. Bräger, M.L. Burt, A. Diederichs, A. Gallus, A. Herrmann, J.C. Koblitz, L. Thomas, U.K. Verfuss, L. Wollheim. 2013. **Temporal trend and seasonal dynamics of harbour porpoises in Pomeranian Bight (Baltic Sea).** 27th Annual Conference of the European Cetacean Society. Setubal, Portugal

- Brundiers, K., K. Schmuck, M. Kost, M. Jabbusch, L. Thomas, H. Benke, J.C. Koblitz. 2013. **Which one is the best? Comparing four different static acoustic monitoring devices.** 27th Annual Conference of the European Cetacean Society. Setubal, Portugal

- Carlström, J., M. Amundin, L. Thomas, J. Tougaard, J. Teilmann, J.C. Koblitz, N. Tregenza, I. Carlen, L. Kyhn, D. Wennerberg, O. Loisa, I. Pawliczka, A. Ikauniece, I. Jussi, Š. Aleksej. 2013. **SAMBAH - Static Acoustic Monitoring of the Baltic Sea Harbour Porpoise.** 27th Annual Conference of the European Cetacean Society. Setubal, Portugal

- Courtene-Jones, W. H. Nuuttila, K. Brundiers, J.C. Koblitz. 2013. **Comparison of broadband hydrophone and C-POD data logger recordings of wild bottlenose dolphin (*Tursiops truncatus*) echolocation clicks.** 27th Annual Conference of the European Cetacean Society. Setubal, Portugal

- Herrmann, A., V. Kosarev, S. Bräger, L. Wollheim, A. Gallus, A. Diederichs, L. Thomas, M.L. Burt, H. Benke, J.C. Koblitz. 2013. **On the way to long-term acoustic monitoring: How to compare C-POD and T-POD data from a low-density area.** 27th Annual Conference of the European Cetacean Society. Setubal, Portugal

- Gonzalez-Terrazas, T.P., J.C. Koblitz, M. Tschapka, T. Fleming, R. Medellín, H.-U. Schnitzler, E.K.V. Kalko. 2012. **How nectar-feeding bats find food: Echolocation behavior of *Leptonycteris yerbabuenae* during foraging at cactus flowers.** 14th Congress of the International Society for Behavioral Ecology. Lund, Sweden

- Carlström, J., M. Amundin, L. Thomas, J. Tougaard, J. Teilmann, J.C. Koblitz, N. Tregenza, I. Carlén, L. Kyhn, D. Wennerberg, O. Loisa, I. Pawliczka, A. Ikauniece, I. Jüssi, E. Visakavičius.2012. **SAMBAH: Static acoustic Monitoring of the Baltic Sea Harbour Porpoise.** 26th Annual Conference of the European Cetacean Society. Galway, Ireland

- Brundiers, K., J. Tougaard, L.A. Kyhn, S. Sveegaard, L. Thomas, J. Teilmann, H. Benke, J. C. Koblitz. 2012. **Acoustic detection functions of C-Pods: estimating probability of detecting harbour porpoise clicks using playback experiments.** 26th Annual Conference of the European Cetacean Society. Galway, Ireland

- Wennerberg, D., L. Thomas, J. Tougaard, N. Tregenza, J.C. Koblitz, L. Kyhn, M. Dähne, J. Teilmann, H. Nuutila, M. Amundin. 2012. **Novel approaches to calculating a c-Pod detection function for the harbor porpoise (*Phocoena phocoena*).** 26th Annual Conference of the European Cetacean Society. Galway, Ireland

- Schaffeld, T., A. Gallus, H. Benke, J.C. Koblitz. 2012. **Feeding at night: diel patterns in echolocation behaviour of harbour porpoises.** 26th Annual Conference of the European Cetacean Society. Galway, Ireland

- Wennerberg, D., L. Thomas, J. Tougaard, N. Tregenza, J.C. Koblitz, L. Kyhn, M. Dähne, J. Teilmann, H. Nuutila, M. Amundin. **Novel approaches to calculating a c-Pod detection**

function for the harbor porpoise (*Phocoena phocoena*). 2011. 5th International Workshop on Detection, Classification, Localization, and Density Estimation of Marine Mammals using Passive Acoustics. Mount Hood, USA

- Wennerberg, D., L. Thomas, J. Tougaard, N. Tregenza, J.C. Koblitz, L. Kyhn, M. Dähne, J. Teilmann, H. Nuutila, M. Amundin. **Novel approaches to calculating a C-Pod detection function for the harbor porpoise (*Phocoena phocoena*).** 2011. 4th International Conference on the Effects of Sound in the Ocean on Marine Mammals. Amsterdam, Netherlands

- Dähne, M., A. Gallus, J.C. Koblitz, U. Siebert, H. Benke. 2011. **Comparison of static acoustic monitoring devices.** 25th Annual Conference of the European Cetacean Society. Cadiz, Spain

- Hansen, S., U. Verfuss, A. Gallus, K. Krügel, J.C. Koblitz, H. Benke. 2011. **From T-Pods to C-Pods – An Approach towards data comparability.** 25th Annual Conference of the European Cetacean Society. Cadiz, Spain

- Koblitz, J.C., P. Stilz, W. Plästerer, A. Denzinger, H.-U. Schnitzler. 2009. **Scanning movements during and between sound emission in Greater Horseshoe bats (*Rhinolophus ferrumequinum*) approaching a 16 microphone array.** 5th Animal Sonar Symposium. Kyoto, Japan

- Stilz, P., W. Pflästerer, J.C. Koblitz, H.-U. Schnitzler. 2006. **Reconstruction of bat sonar beams using a multi-channel microphone array.** 4th Joint Meeting of the Acoustical Society of America and the Acoustical Society of Japan. Honolulu, USA