# Echolocation behavior and source level reduction in the echolocating bats *Myotis myotis* and *Eptesicus fuscus* when catching airborne targets of different strength

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for Maya & Sebastian

and for my parents Gertraud & Hartmut

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# 1 Summary

The narrow space passive gleaning forager *Myotis myotis* and the edge space aerial forager *Eptesicus fuscus* were trained to catch mealworms or reflectors, small point-like targets of differing target strength, in the aerial-hawking mode and their motor and echolocation behavior was recorded and described. It was assumed that the comparison of the approach behavior of the two ecologically different species confirms the hypothesis that the sound pressure level (SPL) reduction during the approach, a behavior that is reported for all bats, has the function to adjust the relation of the perceived emission and echo SPL in a range where the ranging performance of bats is optimal. The motor behavior during prey capture was rather similar in both species, and both species formed a pouch with wing and tail membrane when catching prey. According to changes in echolocation behavior the behavioral phases search flight, initial approach, and terminal approach with buzz 1 and buzz 2 were discriminated to describe a capture event. *Eptesicus fuscus* used search signals with a source level (SL) of about 96 dB re 1 m which were much louder than those of *Myotis myotis* which had a search SL of about 80 dB re 1 m. These values correspond to an emission SPL at the ears of about 106 dB and 90 dB. When approaching the target both species reacted in a similar way with a reduction of sound duration, pulse interval, and SPL. The start of the approach or the reaction distance which was determined as the distance where these reactions started depended in both species on the target strength (TS) and decreased with lower TS. With *M. myotis*, the approach began immediately after the calculated target detection at a target distance of 103 cm (for the mealworm target) and a distance of 125 cm (for the reflector target) - each with an echo SPL of approx. 20 dB. In *E. fuscus*, which emit search signals with a SL 16 dB higher than that of *M. myotis*, the calculated detection distances were distinctly further out than the begin of the approach reaction as defined above. The reaction distance also differed according to the different target strength of the prey items between 132 – 173 cm. The start of the approach was triggered when the echo level of the three target types surpassed 35.3 dB, 38.5 dB and 42.3 dB SPL (referring to the emission SPL at the microphone). With the begin of the approach both bats started with the reduction of the emission SPL. *M. myotis* reduced the SPL from about 90 dB to about 75 – 70 dB at the end of buzz 1 at a distance to the target of 17 – 19 cm thus producing echo SPLs of 37 – 35 dB whereas *E. fuscus* where buzz 1 ended rather early at distances around 50 cm reduced the SPL from 106 dB to between 95 – 88 dB emission SPL thus producing echo SPLs between 46 –

35 dB. In *M. myotis* with its low search SL the reduction rate for the emitted signals was with 3.3 and 3.2 dB/hd (hd = half distance) rather low which resulted in a distinct echo increase during the approach to about 40 dB. In the louder *E. fuscus* the reduction rate was rather high with 11.3, 10.5, and 6.5 dB/hd at the three targets which kept the echo level constant near about 40 dB throughout the whole approach. These results support the working hypothesis of this thesis that the adjustment behavior of bats approaching prey is adapted for a precise ranging performance and depends in each species on the echolocation conditions of their ecological niche. Bats with high search SL have high reduction rates during the approach and keep the echo SPL rather constant near 40 dB whereas bats with low search SL have lower reduction rates of the emitted signals so that the echo SPL is raised during the approach to about 40 dB. The measured data do not allow making exact estimations about the perceived SPL ratios at the cochlea since it is not known how the middle ear reflex effects the auditory input during the approach.

# 2 Zusammenfassung

*Myotis myotis* (Große Mausohr Fledermaus) aus der Gilde der „narrow space passive gleaning forager“ und *Eptesicus fuscus* (Große Braune Fledermaus) aus der Gilde der „edge space aerial-hawking forager“ wurden trainiert, kleine Punktziele wie Mehlwürmer oder kleine Reflektoren mit unterschiedlicher „target strength“ im „aerial-hawking mode“ zu fangen. Dabei wurden das motorische Verhalten und das Echoortungs-Verhalten erfasst und beschrieben. Ausgangspunkt der Arbeit war die Hypothese, dass der Vergleich des Annäherungsverhaltens der beiden ökologisch unterschiedlichen Arten zeigt, dass die bei allen Fledermäusen beobachtete Reduktion des Schalldruckpegels (SPL) während der Annäherung das Verhältnis von wahrgenommenem Aussende- und Echo-SPL so einstellt, dass es für die Entfernungsmessung optimal ist. Das motorische Verhalten beim Beutefang war bei beiden Arten ähnlich, und beide bildeten mit Flügel- und Schwanzmembran eine Tasche, mit der die Beute gefangen wurde. Entsprechend von Veränderungen im Echoortungsverhalten wurden die Phasen Suchflug, initiale Annäherungsphase und terminale Annäherungsphase mit Buzz 1 und Buzz 2 unterschieden, um einen Fangvorgang zu beschreiben. *Eptesicus fuscus* benutzte im Suchflug Laute mit einem source level (SL) von etwa 96 dB re 1 m, die deutlich lauter waren als die von *Myotis myotis*, die im Suchflug einen SL von etwa 80 dB re 1 m einstellte. Diese Werte entsprechen einem an den Ohren anliegenden Emissions-SPL von etwa 106 dB und 90 dB. Bei der Annäherung an das Ziel reagierten beide Arten in ähnlicher Weise mit einer Verringerung der Lautdauer, des Lautabstands und des SPL. Der Beginn der Annäherung oder die Reaktionsdistanz, die als die Entfernung definiert wurden, bei der diese Reaktionen einsetzten, war bei beiden Arten von der „target strength“ abhängig und nahm mit geringerer TS ab. Bei *M. myotis* begann die Annäherung unmittelbar nach der berechneten Detektionsdistanz in einer Entfernung von 103 cm (beim Mehlwurm) und einer Entfernung von 125 cm (beim Reflektor) - jeweils bei einem Echo-SPL von ca. 20 dB. Bei *E. fuscus*, die Suchlaute mit einem SL aussendet, der 16 dB höher ist als der von *M. myotis*, waren die berechneten Detektionsdistanzen deutlich größer als die Reaktionsdistanzen, die entsprechend der unterschiedlichen TS der Beutetiere zwischen 132 – 173 cm variierten. Der Beginn der Annäherung wurde ausgelöst, wenn der Echopegel der drei Ziele 35.3 dB, 38.5 dB und 42.3 dB SPL (bezogen auf den Aussende-SPL am Mikrofon) überstieg. Mit dem Beginn der Annäherung begannen beide Fledermäuse mit der Reduktion des Aussende-SPL. *M. myotis* reduzierte den Schalldruckpegel von ca. 90 dB

auf ca. 75 – 70 dB am Ende von Buzz 1 in einer Entfernung von 17 – 19 cm zum Ziel und erzeugte damit Echo-SPLs von 37 – 35 dB, während *E. fuscus*, bei welcher der Buzz 1 recht früh in einer Entfernung von ca. 50 cm endete, von 106 dB Aussende-SPL in der Suchphase auf SPLs zwischen 95 – 88 dB reduzierte und damit Echo-SPLs zwischen 46 – 35 dB erzeugte. Bei *M. myotis* mit seinem niedrigen SL in der Suchphase war die Reduktionsrate für die emittierten Signale mit 3.3 und 3.2 dB/hd eher gering, was zu einem deutlichen Echoanstieg während der Annäherung auf etwa 40 dB führte. Bei der lauteren *E. fuscus* war die Reduktionsrate mit 11.3, 10.5 und 6.5 dB/hd bei den drei Zielen recht hoch, was den Echopegel während des gesamten Anflugs konstant bei etwa 40 dB hielt. Diese Ergebnisse stützen die Hypothese dieser Arbeit, dass bei Fledermäusen die Regulierung von Aussende- und Echo-SPL beim Anflug auf Beutetiere so eingestellt wird, dass eine präzise Entfernungsmessung möglich ist. Die Reduktionsraten des SL jeder Art hängen dabei von den an ihre ökologische Nische angepassten Echoortungsbedingungen ab. Fledermäuse mit hohem SL im Suchflug zeigen hohe Reduktionsraten während des Anflugs und halten den Echo-SPL relativ konstant bei 40 dB, während Fledermäuse mit niedrigerem SL im Suchflug geringere Reduktionsraten der ausgesendeten Laute aufweisen, sodass der Echo-SPL während des Anflugs auf etwa 40 dB angehoben wird. Die in dieser Arbeit erhobenen Daten ermöglichen keine genauen Abschätzungen über die wahrgenommenen SPL-Verhältnisse an der Cochlea, da nicht bekannt ist, wie der Mittelohrreflex den auditiven Input während des Anflugs zusätzlich beeinflusst.

# 3 List of abbreviations

| | |
|---|---|
| AGC | automatic gain control |
| ANOVA | analysis of variance |
| A/D | analog to digital |
| B&K | Brüel&Kjaer |
| CF-FM | constant frequency-frequency modulated<br>here, the echolocation signal consists of a constant frequency component followed by a frequency modulated sweep |
| d | distance |
| dB | decibel |
| df | degrees of freedom |
| e.g. | “exempli gratia” (Latin), for example |
| et seq. | “et sequens” (Latin), and following |
| FFT | fast Fourier transformation |
| Fig. | figure |
| FM | frequency modulated |
| hd | halving distance |
| i.a. | “inter alia” (Latin), among others |
| i.e. | “id est” (Latin), that is |
| INAT | intensity analysis tool |
| IR | infrared |
| kHz | kilohertz |
| max. | maximum |
| MEM | middle ear muscle |
| min. | minimum |
| mw | mealworm |

| | |
|---|---|
| PD | pulse duration |
| PI | pulse interval |
| p. | page |
| re | relative |
| resp. | respectively |
| rms | root mean square |
| s.d. | standard deviation |
| SL | source level |
| SPL | sound pressure level |
| t | time span |
| Telemike | telemetry microphone |
| TS | target strength |
| TTL | transistor-transistor Logistic |
| TWT | two-way travel (time) |
| v | flight speed |
| vs. | versus |

# 4 Echolocation behavior and source level reduction in the echolocating bats *Myotis myotis* and *Eptesicus fuscus* when catching airborne targets of different strength

## 4.1 Introduction

With about 1400 species worldwide, bats (Chiroptera) represent almost a quarter of all extant mammals (Wilson and Mittermeier, 2019). Nearly 1200 species use laryngeal echolocation and emit ultrasonic echolocation signals. For spatial orientation and prey acquisition they analyze the echoes returning from targets and structures in the environment (Griffin, 1958; Griffin et al., 1960; Neuweiler, 1990; Schnitzler and Kalko, 2001; Schnitzler et al., 2003; Denzinger and Schnitzler, 2013). Dependent on the type of prey and the clutter situation in the foraging habitat bats need to solve different echolocation tasks. Bats foraging in similar clutter conditions and with similar foraging modes have to solve similar tasks and are therefore assigned to the same functional groups or guilds (Schnitzler and Kalko, 2001; Schnitzler et al., 2003; Denzinger and Schnitzler, 2004; Denzinger and Schnitzler, 2013).

One species of this study, *M. myotis*, belongs to the guild of **narrow space passive gleaning foragers**. Members of this guild mainly forage for prey which is positioned on surfaces. Prey echoes are therefore hidden in clutter echoes so that prey cannot be detected and localized by echolocation. Passive gleaners use prey-generated cues to detect and localize their prey (Schnitzler et al., 2003; Denzinger and Schnitzler, 2004; Denzinger and Schnitzler, 2013). *M. myotis* for instance forages in the gleaning mode for arthropods (Kolb, 1958; Arlettaz, 1996; Güttinger, 1997) moving in freshly mowed meadows or on vegetation foliage by listening for prey-generated sounds (Arlettaz, 1996; Güttinger, 1997; Arlettaz et al., 2001; Zahn et al., 2005). However, it has also been described that greater mouse-eared bats hunt for cockchafers in the aerial-hawking mode (Arlettaz, 1996; Güttinger, 1997), though it is not clear whether these bats found the cockchafers by echolocation or simply by listening to the sounds generated by the moving wings. In *M. myotis* which were trained to glean mealworms from a dish offered on the ground of a flight room three phases of the foraging behavior were discriminated: search flight, passive approach and active approach (Budenz et al., 2018). During search flight *M. myotis* flew in large circles over the feeding area at average flight height between 0.6 and 1.4 m and average flight speed between 3 and 4.2 m/s. After prey

detection by passive listening the bats reduced flight height down to only 0.2 – 0.4 m above the ground. The active approach was initiated by a reduction of SPL, PD and PI and signals were emitted in groups.

In laboratory studies *M. myotis* could also be trained to use the active aerial-hawking mode to intercept airborne targets. Deutschmann (1991) describes that bats hunting in the active mode reduced sound duration and pulse interval after the detection of prey and emitted echolocation calls in small groups containing 2 to 5 calls and shortly before reaching the prey they emitted a long terminal group consisting of buzz 1 and buzz 2. A similar approach behavior is already known from aerial-hawking bats. In gleaning *M. myotis* source level (SL) is kept at a low level in order to get as little background noise as possible (Arlettaz et al., 2001; Russo et al., 2007) which enables passive acoustic hearing of prey generated noise.

The other species, *E. fuscus*, belongs to the guild of **edge space aerial foragers**. Bats belonging to this guild are adapted to forage for aerial prey in open and edge space habitat (Denzinger and Schnitzler, 2013.). Typical foraging areas are grassland, parks sometimes lined by trees, wide lanes in wooden areas, or around street lamps (Surlykke and Moss, 2000). *E. fuscus* hunts in different heights from a few meters above the ground up to a height little higher than the tree tops or as high as the street lamps (Surlykke and Moss, 2000). It has also been observed to fly on stereotypical flight paths in familiar areas. When hunting *E. fuscus* scan their surroundings by aiming its sonar beam at relevant objects (Hulgard et al., 2016). When searching for prey they emit downward sweeping echolocation calls with several harmonics with durations between 2 to 4 ms in the laboratory (Surlykke and Moss, 2000; Saillant et al., 2007) and longer search calls in the field (Novick, 1971; Kurta and Baker, 1990; Surlykke and Moss, 2000; Hulgard et al., 2016). As other vespertilionids they operate with the first harmonic which extends from around 48 down to 27 kHz (Fenton and Bell, 1981; Kurta and Baker, 1990). The search signals consist of two parts: A steeply frequency-modulated broadband initial part for localizing and classifying background and a shallowly modulated narrowband terminal part which is well-suited for insect detection at intermediate range (Denzinger and Schnitzler, 2013).

In bats using the aerial-hawking mode the echolocation behavior is described by two phases. In search phase bats search for airborne prey with echolocation calls which are emitted at a

low repetition rate. After the detection of prey bats switch to the approach phase and emit approach signals which are directed towards the prey during the entire insect pursuit (Griffin et al., 1960). The approach sequences are characterized by a reduction of pulse duration (PD) and pulse interval (PI) (Griffin et al., 1960; Schnitzler et al., 1987). Signals are often emitted in groups with an increasing number of pulses as bats close in on the prey. The terminal group of the approach phase is called buzz. In foraging vespertilionid bats, it consists of buzz 1 with continually reduced PD and PI and buzz 2 with minimal PD and constant PI (Griffin et al., 1960; Kalko and Schnitzler, 1989; Melcón et al., 2007; Melcón et al., 2009). In contrast to aerial-hawking bats, gleaning bats do not emit such a distinct terminal group with buzz 1 and buzz 2 when approaching a food site (Thies et al., 1998; Arlettaz et al., 2001; Schnitzler and Kalko, 2001; Budenz et al., 2018).

The SL of search signals of foraging bats differs according to the preferred foraging habitat type. SLs (re 1 m) between 111 – 101 dB have been reported for open and edge space foragers (Holderied and v Helversen, 2003; Surlykke and Kalko, 2008; Stilz and Schnitzler, 2012). Additionally, bats dynamically adjust source levels to conditions in their environment (Jakobsen et al., 2013). Hulgard et al. (2016) stated that SL estimates based on field recording of bats foraging in open space are 15 – 20 dB more intense than that based on laboratory data. E.g., in *E. fuscus* foraging in open areas SLs around 111 dB have been measured (Hulgard et al., 2016) while in flight rooms *E. fuscus* emitted signals with a SL near 96 dB rms re 1 m (Koblitz et al., 2010; Weiss, 2021). In bats foraging in narrow space the SL is much lower. In *M. myotis* searching for rustling prey in a flight room 76 dB were measured (Budenz et al., 2018).

Bats approaching a stationary (landing site, obstacle) or moving target (airborne insect) reduce in the approach phase the sound pressure level (SPL) of the emitted calls most probable to adjust the relation of the perceived emission and echo SPL in a range where ranging performance is optimal (Denzinger and Schnitzler, 1994, 1998; Budenz et al., 2018). Reduction rates of approximately 6 dB per halving distance (hd) varying within a range of 4 – 9 dB/hd have been reported so far (Griffin, 1958; Jen and Kamada, 1982; Kobler et al., 1985; Hartley et al., 1989, 1992 a, b; Tian and Schnitzler, 1997; Boonman and Jones, 2002; Hiryu et al., 2007, 2008; Melcón et al., 2007, 2009; Saillant et al., 2007; Brinklov et al., 2009, 2010; Koblitz et al., 2011; Norum et al., 2012; Budenz et al., 2018). This behavior is quite common

in all echolocating bats. It has been assumed that the SPL reduction compensates the increase in echo SPL at decreasing distance between bat and target.

The fact that loud bats should react in a different way than bats with low SL to adjust an optimal ratio of perceived emission and echo SPL has not been addressed so far. When closing in on the prey bats emitting high SLs have to deal with very high perceived emission SPLs even after a reduction by the middle ear muscle reflex (MEM reflex). On the other hand, the low emission SLs of narrow space bats result in weak echo SPLs. How such differences effect the adjustment of the emission and echo levels during approach has not yet been studied.

Several hypotheses have been published which describe and try to understand the regulation of the echo SPL during approach. Kick and Simmons (1984) performed an echo detection experiment in which stationary bats, sitting on a platform, had to detect electronically simulated targets at different distances. They explain the result of a rising auditory threshold by 11 dB/hd at reduced distances with the hypothesis of an "automatic gain control" (AGC). They suggest that the MEM reflex has the function of an AGC-mechanism by increasing the auditory threshold of incoming echoes by 11 dB/hd to compensate for the increase of echo SPL of 12 dB/hd in point targets. However, the bats were stationary in this experiment and did not reduce the emission SPL so that the postulated effect that bats keep the perceived echo SPL constant would only reflect the action of the MEM reflex. Kobler et al. (1985) published another hypothesis. *Pteronotus parnellii* sitting on a pendulum and swinging towards a wall compensated the echo SPL increase of 6 dB/hd with a decrease of emission SPL by 6 dB/hd. They explain this behavior with the term "intensity compensation". A bat compensates for the increase of echo SPL with a reduction of emission SPL. However, the authors did not consider the middle ear reflex and showed therefore that the echo level is kept constant at the bat's ear. The last hypothesis, finally, is a combination of the further two. Hartley (1992 a, b) and Hartley and colleagues (1989) suggest that bats use a "dual component system for stabilization of the perceived echo-amplitude" by reducing the emission SPL and rising the auditory perception threshold by the MEM reflex. Thus, a stationary bat reduced the emission SPL by 6 dB/hd while a point target with an increase in echo SPL by 12 dB/hd was approaching. Additionally, the hearing threshold increased by 7 dB/hd. Thus, again, the perceived echo SPL would be kept constant. In summary, all mechanisms induce that bats

somehow keep the echo SPL constant at a reference level with a closed loop or feedback control system. This suggests that they employ a feedback control mechanism similar to that which is used for Doppler-shift compensation in flutter-detecting foragers. Doppler-shift compensating bats keep the echo frequency from larger stationary targets constant at a reference frequency by lowering the emission frequency. Thereby they compensate for the positive Doppler-shifts produced by their own flight movement (Schnitzler, 1967; Schnitzler and Denzinger, 2011).

The suggested closed loop control systems for regulation of emission SPL have been questioned by several other studies. A compensation system implies that the distance dependent echo SPL increase rates of different targets are compensated by corresponding decrease rates of the emission SPL. Furthermore, the SPL reduction should start at larger target distances with a loud target and therefore higher TS than with smaller targets with lower TS. Published studies, however, support these predictions only partly. An emission SPL reduction rate of around 6 dB/hd seems to be common independent of target properties and thus does not support the hypothesis of the closed loop control system. *Myotis daubentonii* approaching various objects with different reflection properties (spheres of different size and mealworm) showed a rather stereotyped SPL reduction rate of 4 dB/hd independent of the targets' TS (Boonman and Jones, 2002). Thus, the authors denied a closed loop control system for regulation of SPL reduction. Furthermore, the existing large fluctuations (up to 12 dB) in source level within sound groups found in *Eptesicus fuscus* (Koblitz et al., 2010) also do not support a tightly coupled feedback control system. Recently, Budenz and colleagues (2018) showed that passive gleaning *M. myotis* approaching feeding dishes with different target strengths also reduce the emission SPL but at different reaction distances and reduction rates which contradicts a closed loop control system for intensity compensation. Further, Stidsholt et al. (2020) showed that *Hipposideros patti* and *Hipposideros armiger* did not compensate for different target size in a one to one dB fashion when landing on spheres, also suggesting that bats do not seek to stabilize echo levels at a constant value. Budenz et al. (2018) proposed a different function of emission level adjustment during approach. Supported by neurophysiological (Berkowitz and Suga, 1989; Hechavarria et al., 2013) and behavioral studies (Denzinger and Schnitzler, 1998) which show, that for an optimal processing of the pulse-echo pairs the ratio of the sound pressure levels (SPLs) of the emitted signal and the returning echoes have to be within an optimal processing range of the bats' auditory system,

Budenz et al. (2018) assume that the observed changes in emission SPL during the approach to targets have the function to adjust the ratio of pulse-echo pairs to meet this optimal range.

Most data on reduction of emission SPL during an approach are from landing bats which means that they approached a large extended target. The SPL of echoes returning from such targets increases with about 6 dB/hd during approach due to spherical sound propagation so that the echo SPL at the bats' ear would be kept constant if a bat lowers the emission SPL with 6 dB/hd. During the approach to a small target, e.g., an insect or a small reflector, the echo SPL rises with about 12 dB/hd. The few data on this echolocation task give no clear answer how bats adjust emission and echo levels in this situation. *Myotis daubentonii* approaching small objects showed a rather stereotyped SPL reduction rate of 4 dB/hd (Boonman and Jones, 2002) and two individuals of *Eptesicus fuscus* catching a suspended microphone adjusted the emission level in different ways (Saillant et al., 2007). One individual kept the echo level constant at the microphone corresponding to a decrease rate of 6 dB/hd whereas in the other bat the reduction rate at the microphone indicates a decrease rate of the emission SPL of distinctly larger than 6 dB/hd.

For a better understanding of the mechanisms which regulate the reduction of emission SPL and the adjustment of echo SPL in bats approaching small airborne prey I trained the narrow space passive gleaning forager *Myotis myotis* and the edge space aerial forager *Eptesicus fuscus* to catch small point-like targets of differing target strength in the aerial-hawking mode and described their motor and echolocation behavior. A main aim of the study was to describe the echolocation behavior of the foraging bats and especially to determine the change of the auditory input during the approach as described by the change of the SPL of the emitted signal and of the returning echo measured with an on-board microphone near the base of the pinna. All SPL values mentioned in this study are related to this microphone.

Due to the fixed position of the microphone at the head it is possible to use the microphone SPL as an estimate for the source level (SL) related to 10 cm in front of the bat. I will compare my data with the changes in emission SL and echo level of the same species when approaching extended landing sites, which have already been published (Koblitz et al., 2011; Budenz et al., 2018). If approaching bats would compensate the increase in echo SPL in a closed loop feedback control system I would expect distinct differences in the adjustment

behavior between approaches to targets of different strength and also between approaches to an extended and to a point target with an echo increase of 6 resp. 12 dB/hd.

I am especially interested to compare the SL reduction rate of the two species and its effect on the echo SPL during approach. I assume that *E. fuscus* with its higher SL will react in a different way as *M. myotis* with its low SL to reach the optimal ratio between emission and echo level for precise range measurements. This would indicate that not only the different SLs of the search signals but also differences in the SL reduction during the approach reflect that *E. fuscus* and *M. myotis* occupy different ecological niches.

## 4.2 Material and Methods

### 4.2.1 Bats

#### 4.2.1.1 Experiments with *Myotis myotis*

The experiment was conducted with four male greater mouse-eared bats, *M. myotis* (Fig. 1), (Borkhausen, 1797), at the Institute for Neurobiology of the University of Tübingen, Germany, from June 2012 to July 2013. They were housed in the institute's animal facility under standardized conditions (24 ± 5°C temperature, 65 ± 5% humidity, a reversed light:dark cycle of 15:9 hours). Bats always had free access to water and during the week were only fed during experimental session. The food supply consisted of mealworms and mealworm beetles (*Tenebrio molitor*) supplemented with crickets and locusts every view weeks. Additionally, bats got vitamins (Efaderm® liquid, aristavet GmbH & Co, Germany) and minerals (Korvimin®, WDT eG, Germany) every second week. Bats were captured from a large colony roosting in a cave under the license No 55-3/8852.21 from March 03, 2009 of the "Regierungspräsidium Tübingen". All experiments were approved by the "Regierungspräsidium Tübingen" (permit No 35/185.81, ZP 2/08).

**Fig. 1: *M. myotis*, the greater mouse-eared bat.** © Verena Müller

#### 4.2.1.2 Experiments with *Eptesicus fuscus*

The experiment was conducted with two adult female big brown bats, *E. fuscus* (Fig. 2), (Beauvois, 1796) at the Institute for Systems Research of the University of Maryland (USA) in August and September 2013. Bats were collected in Silver Spring, Maryland, on June 4$^{th}$ 2013 and have not been in any experiments before. They were housed with others in a colony room at the University of Maryland under standardized conditions (reversed light:dark cycle of 12:12 hours, 27°C temperature) and had free access to water. Bats were kept on a diet of mealworms (*Tenebrio molitor*). On experimental days bats were first fed during the experimental session and afterwards if necessary. The experiment was conducted under the animal protocol R-13-21 "Multisensory integration for spatial navigation and flight control".

**Fig. 2: *Eptesicus fuscus*, the big brown bat.** © Sonja Sändig

### 4.2.2 Experimental setup

#### 4.2.2.1 Experiments with *Myotis myotis*

The experiment with *Myotis myotis* was conducted in a flight room in complete darkness to prevent the use of vision. Ceiling, walls and ground were covered with sound-absorbing foam to reduce echoes. The room was divided by a black curtain into an experimental area (8*6.1*2 m) where the experiments took place (Fig. 3) and an equipment area (5*6.1*2 m) with most of the recording equipment containing computer, telemetry-antenna, receiver and other devices.

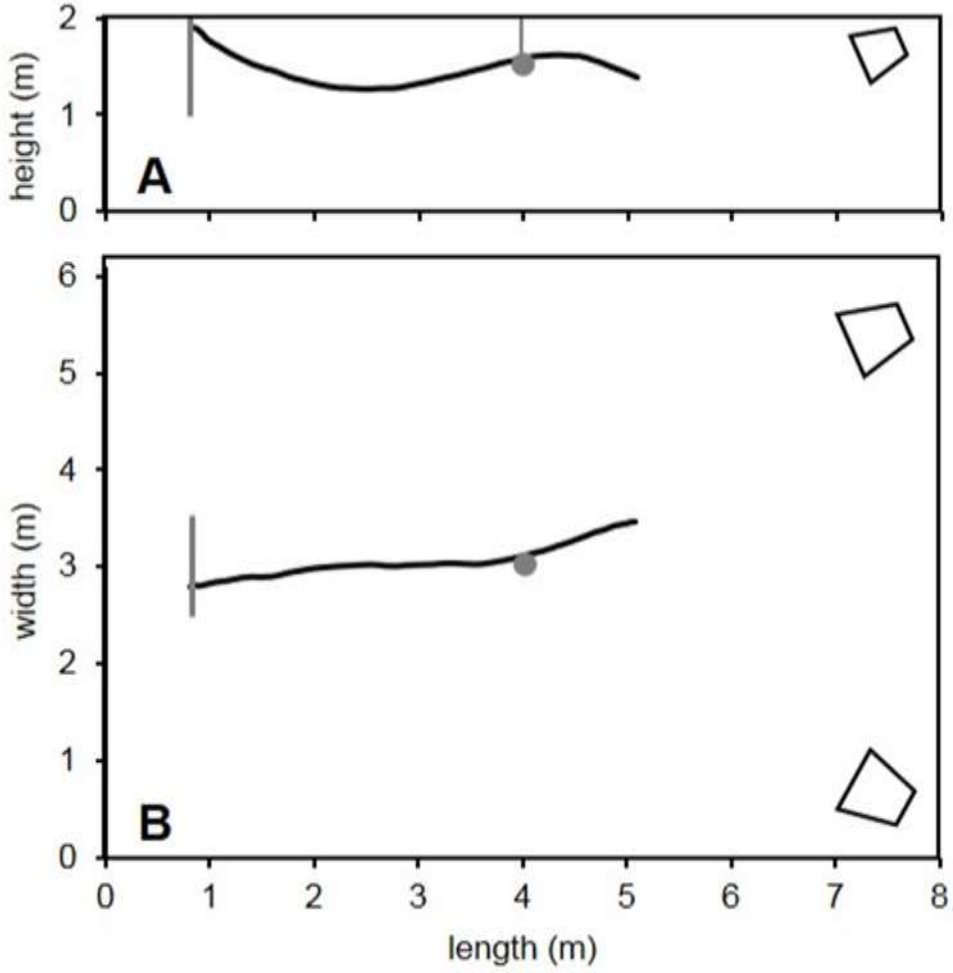



**Fig. 3: Side view (A) and top view (B) of a target approach in the experimental area of the flight room.** The bat flew from the starting grid (grey line) to the tethered target (grey sphere). Two cameras in the upper corners of the experimental area recorded the bats' flights from ahead.

The bats already had experience in behavioral studies. Some years before, they were trained to carry a backpack with a Telemike when searching for and approaching sites with food so that the applied method of data collection wasn't completely new for them (Budenz et al., 2018). However, they never had learned to search for and to approach airborne prey. Bats were trained to wait on a vertical grid (1*1 m) on the far end of the experimental room until the experimenter gave the start signal for a trial. After start the bats had to approach and to catch the target hanging on a string (0.2 mm wire diameter) in the middle of the room (Fig. 3). Since the target was always presented at the same spot with a deviation of only up to 10 cm, I assume that the bats had expectations on target position when performing the trials.
Two different types of targets with different target strength were presented. A mealworm represented the weak target and a globular corner reflector made of aluminum foil represented the loud target. The globular corner reflector had a diameter of 1.8 cm and consisted of 3 perpendicular intersecting flat planes which reflected the echoes directly back towards the bat.

#### 4.2.2.2 Experiments with *Eptesicus fuscus*

The experiment with *Eptesicus fuscus* was also conducted in a flight room (7.5*6.5*2.6 m) in complete darkness. Again, walls and ceiling were covered with sound-absorbing foam and the ground was covered with a curtain. Before starting with the experiment, I reassured myself that bats were eager to approach targets attached to a string (0.2 mm wire diameter) hanging from the ceiling and were able to catch them, also with the recording system (see 4.2.3) on the back. However, a long and special training of the bats turned out not to be necessary for approaching and catching prey and I only had to spend a couple of days to get the bats used to the backpack containing the recording device. Prey was either one single mealworm attached to the end of the string or a cluster consisting of four mealworms. Prey-like targets were self-built cubic plastic reflectors of 1 cm and 2 cm side length with open edges (Fig. 4). However, bats only rarely approached the large reflector, and therefore it was not considered in the analysis. The position of the string was changed every day and in each trial, and one of the three targets (single mealworm, mealworm cluster and reflector) was offered randomly. To keep bats motivated more trials with food rather than with the reflector were conducted. Before the experimenter started the recordings, bats mostly hang on one of the walls or sometimes flew through the room. In those cases where the bats were already flying, the

target was shielded by the experimenter's hands. As soon as the recordings started the bats flew around and searched for the tethered target in the middle of the room. Each trial ended with a successful catch of the presented target or an approach with contact of the string as bats sometimes failed to rib off the mealworms.

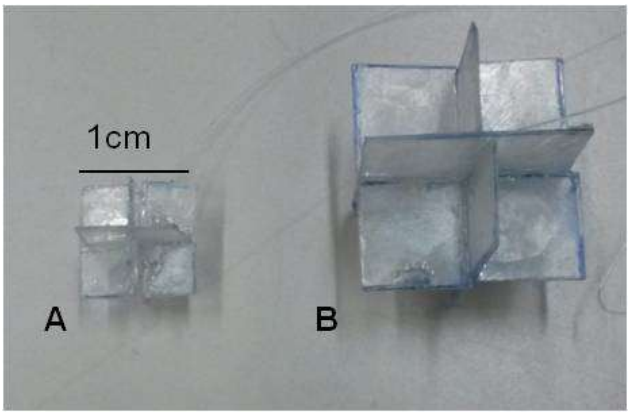



**Fig. 4: Custom-made reflectors with 1 cm (A) and 2 cm (B) side length** used as prey-like targets tethered from the ceiling.

### 4.2.3 Data recording and analysis

In both experiments the bats' echolocation calls were recorded with a small ultrasonic microphone with omni-directional characteristics (Knowles electronics, SPM0204UD5, USA) which was connected to a radio transmitter. The microphone, the FM-transmitter with connected antenna (180 MHz) and the button cell of 1.55 V are referred to as telemetry microphone (Telemike). The overall weight was 3.5 g.

For the calibration of the Telemike I used a custom-made ultrasonic loudspeaker. This speaker was calibrated with a 1/8" Brüel&Kjaer (B&K) microphone Type 4138 with known sensitivity and frequency response and a Wave Analyzer (Rhode & Schwarz, Germany 1030.7500.05). The frequency response curve of the loudspeaker indicated a rather flat response between 15 and 110 kHz with a maximum output at 65 kHz (Fig. 5).

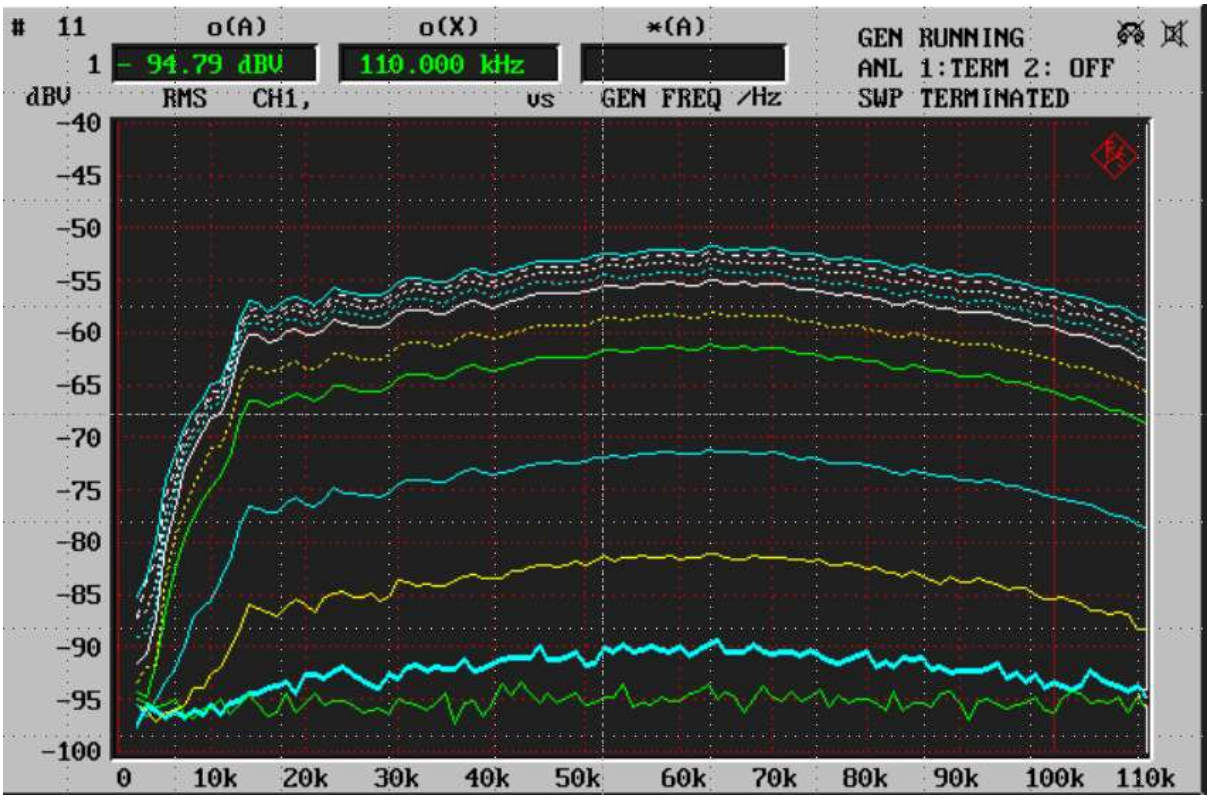
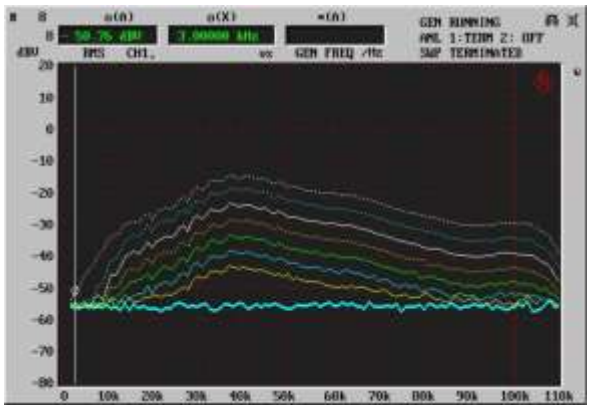



**Fig. 5: Frequency response of the loudspeaker at different SPLs picked up with the B&K.** DC-voltage 170 V, AC-voltage was varied, frequency steps 500 Hz. The colored lines correspond to different SPLs at a distance of 40 cm. From bottom up: turquoise ≙ 61 dB, yellow ≙ 71 dB, blue ≙ 81 dB, green ≙ 91 dB, dotted yellow ≙ 94 dB, white ≙ 97 dB, further curves in steps of 1 dB.

The calibrated loudspeaker was used to determine the frequency response curve and the dynamic range of the two different Telemikes which were used in the *Myotis* or in the *Eptesicus* experiment. In this calibration with the Wave Analyzer I determined the voltage at the output of the FM receiver of the Telemike at different SPL levels at 40 cm in front of the loudspeaker. The output of the FM receiver as displayed in Fig. 6 A and Fig. 7 A was also recorded with the PC tape. Thus it was possible to determine the SPL of Telemike signals that were recorded with the PC tape.

The Telemike used in the *Myotis* experiment had its highest sensitivity at 40 kHz and was 6 dB less sensitive at 20 kHz and 10 dB less sensitive at 100 kHz, resulting in a rather linear frequency response in the frequency range of the *Myotis* calls. Noise level was reached at 60 dB. At 40 kHz the dynamic range curve was linear from the noise level to about at least 100 dB, resulting in a dynamic range of the recording system of at least 40 dB (Fig. 6 A, B).



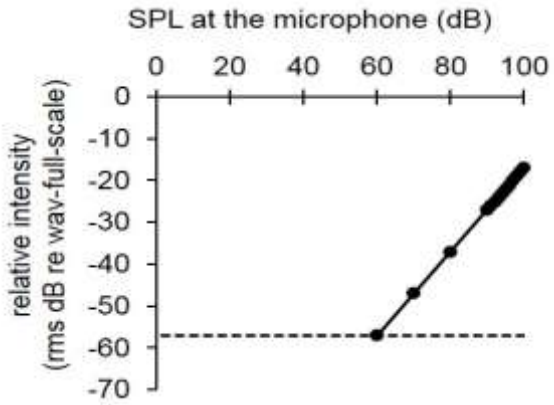



**Fig. 6: (A) Frequency response of the Telemike used for the recordings of *Myotis myotis***, measured with the Wave Analyzer. The colored lines correspond to different SPLs at a distance of 40 cm. From bottom up: turquoise ≙ 60 dB, yellow ≙ 70 dB, blue ≙ 75 dB, green ≙ 80 dB, dotted yellow ≙ 85 dB, white ≙ 90 dB, dotted turquoise ≙ 95 dB, dotted white ≙ 100 dB.

**(B) Dynamic range of the Telemike used in the *Myotis* experiment.** Noise level was reached at 60 dB SPL. The linear dynamic range is at least 40 dB from 60 – 100 dB.

The Telemike used in the *Eptesicus* experiment had its highest sensitivity also at 40 kHz. It was 5 dB less sensitive at 20 kHz and 13 dB less sensitive at 100 kHz. Noise level was reached at 40 dB and dynamic range curve was linear up to 79 dB. The recording system had a dynamic range of not more than 39 dB (Fig. 7 A, B).

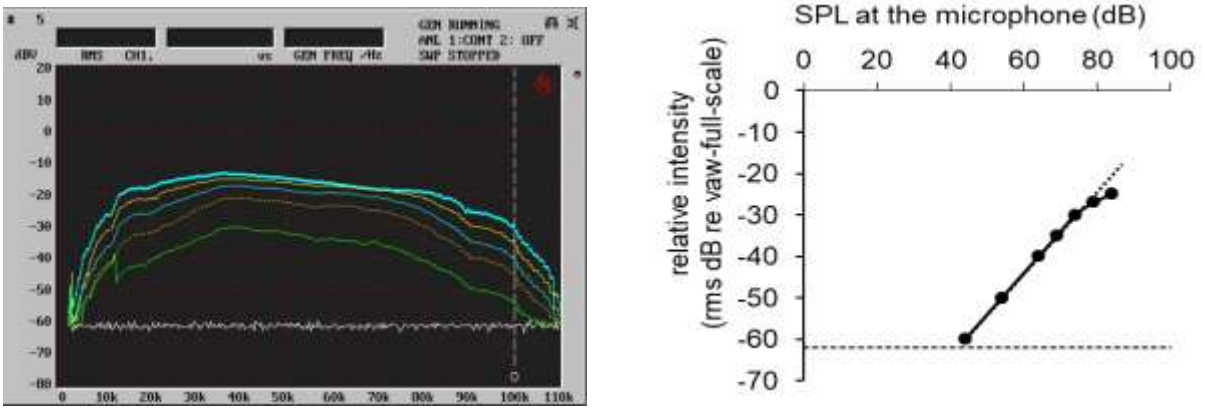



**Fig. 7: (A) Frequency response of the Telemike that was used for *Eptesicus* recordings.** The colored lines correspond to different SPLs at a distance of 40 cm. From bottom up: white ≙ 40 dB, green ≙ 70 dB, dotted yellow ≙ 80 dB, blue ≙ 85 dB, yellow ≙ 90 dB, turquoise ≙ 95 dB.

**(B) Dynamic range of transmission path of the Telemike used in the *Eptesicus* experiment.** Noise level was reached at 40 dB SPL. The linear dynamic range amounts not more than 39 dB, reaching from 40 dB up to 79 dB.

For sound recordings of flying bats, the Telemike was fixed in a self-made backpack that was attached to the bat with a small rubber band around the neck and stabilized with a drop of skin glue (Mastix Watersoluble, Grimas®, Holland) at a shaved part of the bat's back. The microphone was attached at a connection wire so that it could be positioned on the bats' head, pointing into flight direction. After each recording session, glue and backpack were easily removed with water.

In the *Myotis myotis* experiment two VHF-antennae in the equipment area of the flight room that were connected with a custom-made FM-receiver picked up the radio signals of the Telemike. The transmitted echolocation calls were then digitized (256 kHz, 16 bit) with a custom-made A/D converter (PC tape, Department of Animal Physiology, University of Tübingen, Germany) and stored as wav files. Flight behavior was recorded with an infrared-

video-system (IR CCD Camera, Sanyo, Japan; IR 4.8 mm lenses, TV lens, Germany, 50 half-frames per second) consisting of two cameras that were positioned in the upper corners of the flight room facing the approaching bat. The cameras recorded half-frames (50 Hz) that were illuminated by infrared flashes for 1 ms each. The video data were transferred to two camcorders (DCR-PC8E, Sony Corporation, Japan) and stored on video tapes. Synchronization of sound- and video recordings was possible due to a vertical interval time code (VITC). Digitization and analysis of video recordings were done with commercial software (SimiMotion 6.5, SIMI Reality Motion Systems GmbH). I reconstructed the bats' three-dimensional flight path with a maximum reconstruction error of ± 2.1 cm in flight direction, ± 3.2 cm in width and ± 1.2 cm in height.

In the *Eptesicus fuscus* experiment the bats and their flight behavior were recorded with an IR-high speed-video-system (Miro, M310, Vision Research, AMATEK, USA, 150 frames per second) consisting of two cameras that were positioned in the upper corners of the flight room. Therewith, high quality videos were recorded to visualize the bats' flight behavior. The bat's 3D position, however, was tracked with 16 high speed pure tracking cameras (Vicon, T040, Japan, 300 frames per second) positioned along all four sides of the room, also directly underneath the ceiling. Since these cameras are sensitive to light reflected from infrared-reflecting foil the bat was marked on the wing tips and tail membrane, as well as on the backpack itself, resulting in 5 reflecting points to be tracked. For each frame, the bat's position in the room was calculated as mean of these tracked marks, using Vicon software. A custom-made adder was used for synchronization of video and sound recording systems by adding a TTL pulse to the sound recordings as soon as the video recording was stopped. Both, the data from Vicon and Miro cameras were directly stored in digitized form on the computer.

Flight speed (m/s) of *E. fuscus* was determined from one call position to the next call position or from the position of the last call to the position of the catch (target distance 0 m), respectively. Therefore, I used the common formula $v = s/t$, where $v$ is the flight speed, $s$ is the traveled route and $t$ is the time span.

In both experiments sound recordings were analyzed using a custom-written software (Selena, Animal Physiology, University of Tübingen, Germany). For overview, signals were displayed in color spectrograms (FFT 512, Hann) with a dynamic range of 90 dB. Calls were analyzed

in the precise analysis window (20 ms, FFT 256, Auto padding, 512x512 pixel). The resulting interpolation produced a time resolution of 0.04 ms and a frequency resolution of 250 Hz. The beginning and the end of a call and thus also start frequency and terminal frequency were determined at -10 dB and for buzzes at -6 dB below peak amplitude of the signal. In the *Eptesicus fuscus* experiment, however, start and end of the echoes were always determined at -10 dB. Pulse interval was measured from the beginning of a call to the beginning of the preceding call. When it was not clear whether there was any call between two identifiable calls, pulse interval was not analyzed.

By calculating the two-way travel (TWT) time for the bats' echolocation calls for the complete sequences and comparing it to the PIs and PDs I checked if there is an overlap of the returning echo of a call with an outgoing call in *M. myotis* and *E. fuscus*, respectively. As long as the pulse interval is greater than the TWT time, the situation never arises that a new call is emitted before the echo from the last call arrives at the bat. Likewise, the emitted sound and the returning echo do not overlap if the duration of the sound remains below the two way travel time. Since recorded echolocation calls of *M. myotis* did not show a standard pattern in PD, PI and SPL for the very first centimeters/milliseconds after the bats' start, I only analyzed echolocation calls starting at a target distance of three meters.

For determination of the SPL of signals and echoes at the microphone I used a custom written program (INAT, intensity analysis tool, University of Tübingen, Germany). The signal that is analyzed can be pre-processed by a definable band bass filter. In a wav-file, such as that generated by Selena, the full scale amplitude is defined as ± 1. I calculated the root mean square (rms) amplitude of the echolocation calls and echoes relative to full-scale, after the function ran over the signal using INAT.

The rms amplitude is given by:

$$A_{rms} = \sqrt{\frac{1}{N}}\sqrt{\sum\nolimits_{N} s_n^2} = \sqrt{\frac{1}{T}}\sqrt{\sum\nolimits_{\tau} s(t)^2}\ \sqrt{\Delta(t)} = \sqrt{\frac{E_{eq}}{\tau}}$$

The level L (dB) is defined as:

$$L = 20 * log_{10}(\frac{A_{rms}}{A_{ref}})$$, where $A_{ref} = 1$ here.

Compared to the rms value of a harmonic signal itself, the rms value of the envelope of a harmonic signal is higher by a factor of $\sqrt{2}$ and its level is increased by 3 dB. A permanent sine in a wav-file has an envelope rms amplitude of 1 (= -0 dB) and a signal rms amplitude of 0.7 (= -3 dB re wav-full scale) (Budenz, 2014).

For the determination of the absolute SPL of the Telemike signals recorded with the PC tape I used the following calibration procedure. I generated with the Wave Analyzer and the calibrated loudspeaker sweeps (20 – 100 kHz) of known rms SPL, recorded these sweeps with the Telemike, transmitted them to the receiver, and stored them on the PC tape. With such sweeps it is possible to determine with INAT the absolute SPL from comparable PC tape recordings (Fig. 8). The frequency of 40 kHz was used as reference to determine a relative value (rms dB) for each amplitude in INAT. The input amplitude and reference value could then be used to create calibration curves for the Telemikes used, which can be seen in Fig. 6 B and Fig. 7 B. In the end, the absolute SPL could be determined for each signal that was measured in INAT.

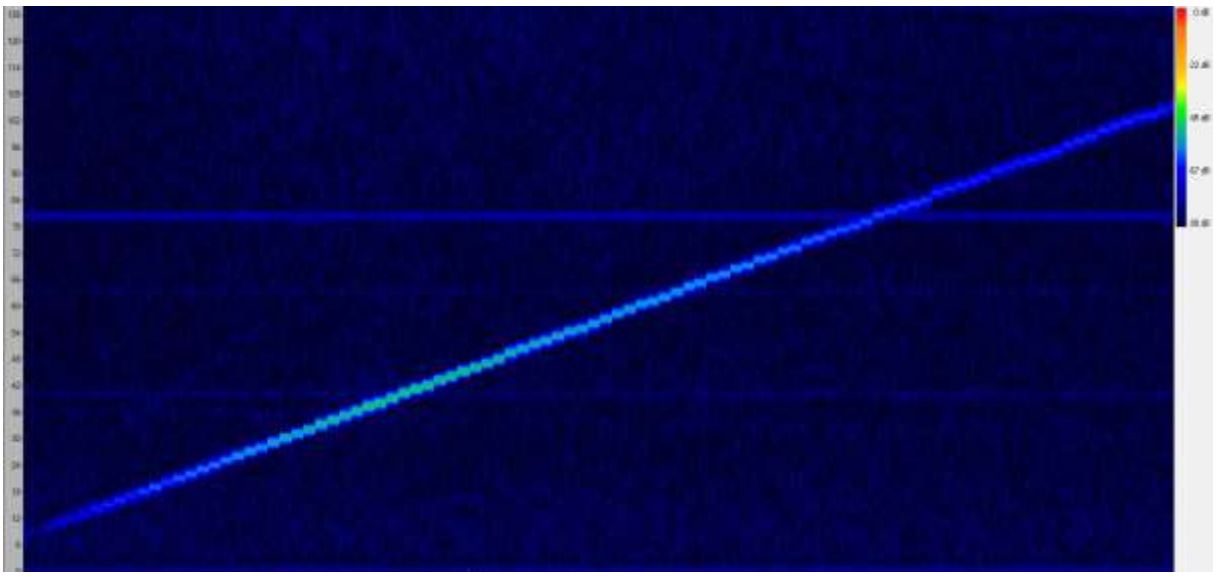


**Fig. 8: Exemplary sweep of 80 dB at the microphone of the Telemike**, generated with the Wave Analyzer and the calibrated loudspeaker, transmitted to the receiver and stored on the PC tape. With such sweeps it is possible to determine with INAT the absolute SPL from comparable PC tape recordings. The x-axis shows the time course of the sweep, the y-axis represents the frequencies from 0 to 128 kHz. The amplitude at the microphone is color-coded: The lighter the louder.

This procedure worked well in the *Myotis* experiment. There, the output of the Telemike receiver was directly fed into the input of the PC tape recording system in a similar way as in the recordings where the Telemike was calibrated (Fig. 8). The same procedure was planned for the *Eptesicus* experiment. However, there, the calibration information was destroyed by a special feature of the synchronization procedure between video and sound recordings in the used equipment. Here, the output of the Telemike receiver was additionally fed through a synchronization unit where the signal was additionally digitized and converted into an analog signal again before it was stored with PC tape. Therefore, I could no more use the same calibration procedure as in the *Myotis* experiment to determine the absolute SPLs of the recorded PC tape signals.

To overcome this fault I used another procedure. I assumed that *E. fuscus* in our experiments emitted search signals which had the same source level (SL) as *E. fuscus* flying under rather similar conditions in other laboratory experiments (Koblitz et al., 2010, 2011; Weiss, 2021). In these studies SLs at 1 m of 94 – 97 dB had been measured which would correspond to a microphone SPL of 104 – 107 dB rms in our experiment. For the calibration I assumed an average SL at 1 m of 96 dB for the search signals which would correspond to a microphone SPL of 106 dB rms in our setting. This gave me the possibility to adjust our measured relative INAT values at the Telemike at the begin of approach to the assumed absolute search signal microphone SPL of 106 dB rms by equating the values of the regression line at the beginning of the approach with the 106 dB.

To calculate the change rate of the SPL with halving or doubling of the distance, I plotted the SPL values in a figure with logarithmic distance scale. In the equations of the corresponding regression lines, I substituted two values for distance, one distance being exactly half the other. The equations were calculated for both distances and the resulting values for each distance were subtracted from each other. This allowed me to calculate the rate of change.

In the *Myotis* experiment the high noise level of 60 dB prevented the recording of echoes from the mealworm and reflector targets due to their low target strength. Thus, echo increase rate could not be measured directly. However, with the known TS, target distance, and SL re 1 m it was possible to calculate a hypothetical echo SPL at each position of call emission with the following formula:

$$echo\ level = SL + C_0 + C_1 * log_{10}\left(\frac{d}{d_0}\right)$$

Here, SL = source level relative to 1 m, $C_0$ = target strength, $C_1$ = slope of the reflection behavior of the target dependent on different distances in decadic logarithm, d = distance at sound emission, $d_0$ = distance for target strength.
Therewith, the echo increase rate could be calculated as change per halving distance (/hd).

The SPL of the emitted signals measured with the Telemike between the ears is lower than the source level (SL) at 10 cm distance in front of the bat due to the directionality of sound emission. For an estimation of the SL I used the approach of Budenz et al. (2018) who used data from Henson (1965) and assumed that the SPL at the Telemike is about 10 dB smaller than the SL measured at 10 cm in front of the bat. With this approach I calculated the SL at 10 cm by adding 10 dB to the microphone SPL, and the SL at 1 m by subtracting 10 dB.

For the *Eptesicus fuscus* recordings I used another Telemike which was more sensitive down to an SPL of 40 dB. Therewith I could record echoes above about 40 dB SPL. Thus, I was actually able to pick up the echoes from the target but with the disadvantage that the emitted signals were mostly in the clipping range of the recording system and emission SPL could not be measured directly. The target echo was identified within the echo train by calculating the two-way travel time of the sound from the bat's position at signal emission to the target. Echo SPL was determined with INAT and the corresponding emission SPL at the microphone was calculated as follows:

$$SL = echo\ level - C_0 - C_1 * log_{10}\left(\frac{d}{d_0}\right),$$

$$emission\ SPL\ measured\ at\ the\ microphone = SL + 10\ dB$$

Again, I could therewith determine the emission SPL decrease rate as the change per halving distance.

### 4.2.4 Definition of beginning of approach and beginning of SPL reduction

The beginning of the approach phase is defined by reactions of the bats to the target, characterized by changes such as shortening of PD and PI. Here I defined the beginning of the approach phase as the beginning of the first call that had shorter pulse duration and pulse interval than the mean values of the search calls minus one standard deviation.

More or less simultaneously call SPL starts to decrease compared to the search phase. For preliminary estimates of the search phase in the *Myotis* experiment means and standard deviations (s.d.) of search signal SPLs were calculated until a target distance of $\geq$ 1.5 m (mealworm) and $\geq$ 1.8 m (reflector). From these values the beginning of SPL reduction was defined as reduction by at least 2*s.d. below the means of the preceding search signals. In consequence, search phase was defined as the range of calls before the beginning of approach (as stated in Table 3). The distances when the approach began according to PI and PD reduction and according to SPL reduction are given relative to the location where bats caught the target (catch = distance 0 m).

In the *Eptesicus fuscus* experiment I could not make a statement about whether the reduction of PD and PI at the begin of the approach phase is accompanied by the begin of a decrease of emission SPL at all, because there were no echoes present in the recordings of the search phase that I defined using the PD and PI.

### 4.2.5 Definition of SPL values used for the description of SPL reduction during an approach

For the description of the SPL reduction during an approach I used the following terms (see Table 3). The mean SPL of the search phase was calculated as the mean SPL at the microphone of all calls per bat that were emitted before approach phase. The mean SPL at the beginning of reaction, at the beginning of buzz 1 and at the end of buzz 1 were also determined on the basis of all data per bat, dependent on each bat's average distance at the beginning of reaction, the beginning of buzz 1 and the end of buzz 1. To determine the mean distance of the end of SPL reduction I first calculated a mean for each bat out of the positions of the last buzz 1 calls and therewith an overall mean, thereby avoiding pseudo-replication. The mean SPL reduction rate (dB/hd) for each bat is the mean that results from the reduction rates of all flights per bat to the respective target, referring to initial approach and buzz 1. In the *Myotis* experiment the absolute value of SPL reduction was calculated from the difference between SPL during search and SPL at the end of approach. Due to the lack of data about SPL of the search phase, in the *Eptesicus* experiment I calculated this value as the difference between the emission SPL calculated from the first echo SPL of the approach and the emission SPL determined from the echo SPL at the end of buzz 1. Furthermore, because I had rather few data points per flight in the *E. fuscus* experiment, I determined the SPL values for each bat directly from the total data points per bat (i.e. from the entity of flights per bat).

### 4.2.6 Ensonification of the targets

The acoustic properties of the chosen targets (mealworms and reflectors) were investigated by ensonification. Target strength is the ratio of sound intensity of the reflected wave in a reference distance (e.g. re 1 m) to the sound intensity at the object (Stilz and Schnitzler, 2012). TS of our objects was determined with white noise signals with frequencies from 30 to 100 kHz. In total, 32 measurements were conducted for each object. In a second step, I used 40 kHz sinusoidal signals to determine how the echo SPL changed with distance. These signals were produced with a calibrated loudspeaker, had an amplitude of 90 dB at a distance of 40 cm and a duration of 1.5 ms. Time delay between the end of the first signal and the begin of the next signal was 30 ms. The recording custom-made ultrasonic microphone was positioned close to the loudspeaker. In order to determine the distance-dependent change in

the echo SPL returning from the objects, I positioned the tethered objects in 5 different distances (40 to 120 cm in steps of 20 cm) to the ensonification tool consisting of loudspeaker and microphone and ensonified the targets from various directions. For each distance, 80 data points were included in the analysis. The echo-amplitude of the targets was analyzed in our custom written program INAT. Thus I got the TS as well as the distance-dependent echo SPL increase rate of the targets (Fig. 9, Fig. 10).

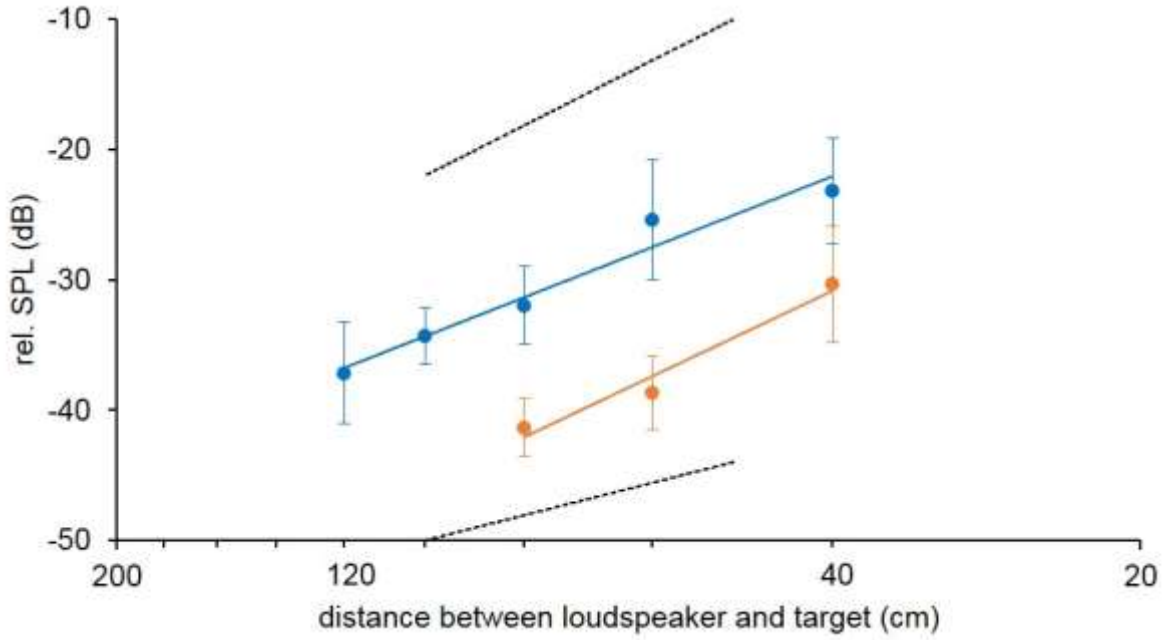



**Fig. 9: Distance related increase in relative echo SPL (mean ± s.d.) of the mealworm and the reflector target of the *Myotis myotis* experiment ensonified with a 40 kHz sinusoidal signal of 90 dB at 40 cm distance.** Targets are color-coded: orange = mealworm, blue = reflector target. The black dashed reference lines indicate slopes of 12 and 6 dB/hd.

Regression equations: mealworm: $y = -16.18\ln(x) + 28.856$, resulting in a relative echo SPL increase of 11.2 dB/hd; reflector target: $y = -13.39\ln(x) + 27.337$, resulting in an increase of relative echo SPL of 9.3 dB/hd.

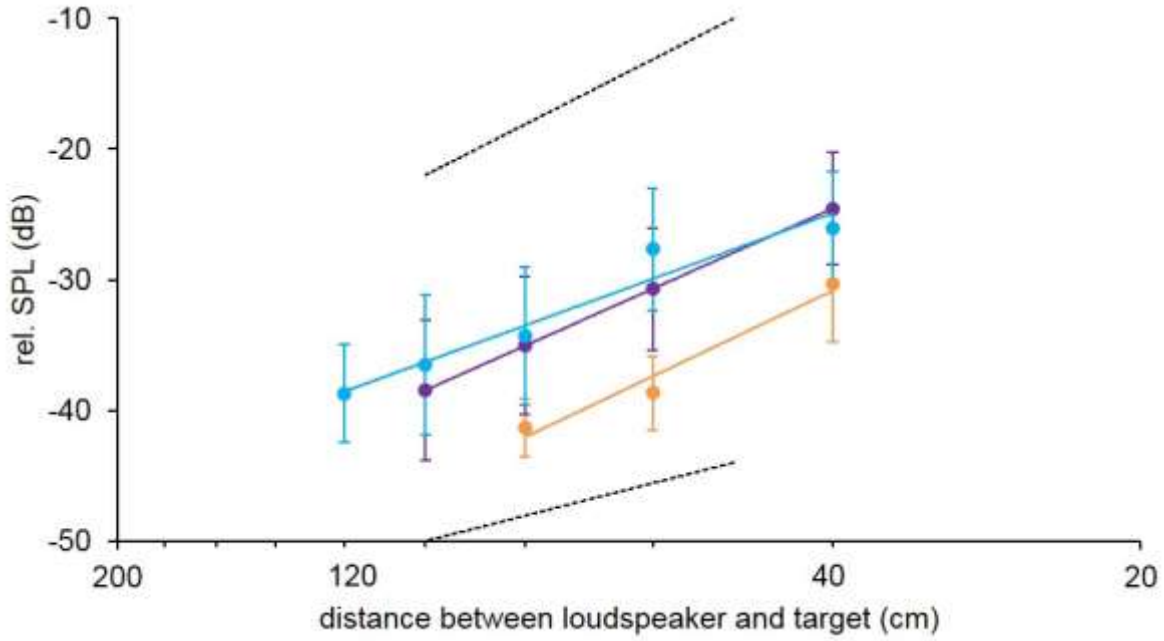



**Fig. 10: Distance related increase in relative echo SPL (mean ± s.d.) of a single mealworm, the cluster of four mealworms and the small reflector target of the *Eptesicus fuscus* experiment ensonified with a 40 kHz sinusoidal signal of 90 dB at 40 cm distance.** Targets are color-coded: orange = single mealworm, violet = four mealworm cluster and blue = reflector target. The black dashed black reference lines indicate slopes of 12 and 6 dB/hd.

Regression equations: single mealworm: y = -16.18ln(x) + 28.856, resulting in a relative echo SPL increase of 11.2 dB/hd; four mealworm cluster: y = -15.18ln(x) + 31.444, resulting in an increase of relative echo SPL of 10.5 dB/hd; reflector target: y = -12.41ln(x) + 20.844, resulting in an increase of relative echo SPL of 8.6 dB/hd.

### 4.2.7 Database and statistics

In the *Myotis myotis* experiment for each of the bats and each target type, four flights were analyzed when approaching and catching the one of the two targets. This resulted in 32 analyzed and synchronized flights. For audio analysis I had a total of 980 echolocation calls available, whereof 539 calls belong to the mealworm trials and 441 calls to the reflector trials. However, it sometimes happened that a call was superposed by background noise potentially occurring by the bat's movement interfering the data transfer from the transmitter to the receiver. With INAT, I could only analyze those calls with a good signal to noise ratio. Besides, SPL was only analyzed in search, initial approach and buzz 1 phases. The course of SPL in buzz 2 was not considered. Other studies (Melcón et al., 2007; Löschner, 2015) let assume another mechanism of signal processing in buzz 2 than in initial approach and buzz 1 because buzz 2 is emitted after the minimal reaction time of 50 ms and the control of approach itself therefore ends with the last signal of buzz 1 (Melcón et al., 2007; Geberl et al., 2015). Thus, I had 407 calls for SPL analysis in the mealworm-situation and 332 calls in the reflector-situation available, resulting in 739 calls.

In the *Eptesicus fuscus* experiment I recorded 50 flights of two *E. fuscus* catching or making contact with the offered targets. For each bat I analyzed 5 flights per target, resulting in 30 analyzed flights, 10 for each target type. In the single mealworm condition 563 calls were included into the sound analysis, in the cluster of four mealworm condition 608 calls, and in the condition with the artificial reflector 588 calls. For SPL analysis I only considered echoes of approach and buzz 1. As I could not identify the target echo of each echolocation call, I only used 79 calls for SPL analysis in the single-mealworm condition, 116 calls in the four-mealworm condition and 143 calls for the reflector condition, resulting in 338 calls in total.

Statistical analyses were performed with Microsoft Excel 2013 (©2012 Microsoft Corporation) and JMP® for windows (13.0.0 ©2016 SAS Institute Inc.). For each analysis, a Shapiro-Wilk test was first performed to check whether the available data were normally distributed or not. In cases of normal distribution parametric tests were applied, otherwise I used non-parametric tests.

In the experiments, paired tests were applied to test the different targets (mealworm vs. reflector and mealworm vs. mealworm cluster vs. reflector, respectively) for significant differences since all target approaches of *Myotis* and *Eptesicus*, respectively, were performed with the same individuals.

In the *Myotis myotis* experiment, I performed 2-factor ANOVAs (with the factors 'bats' and 'targets') and Kruskal-Wallis rank tests, respectively, to test the 4 bats for individual differences. In order to analyze the two targets (mealworm and reflector) regarding any significant differences, t-tests and Wilcoxon signed rank tests were applied. Furthermore, for any significant individual differences, a post hoc test specified which bat differed significantly from the others.
A regression analysis was performed to test whether echolocation parameters depended on target distance. For the parameters emission SPL and echo SPL target distance was logarithmized. 2-factor ANOVAs were furthermore performed to test for differences in the beginning of approach according to reduction of pulse interval and pulse duration and beginning of SPL reduction between targets and bats. By default, p-values $< 0.05$ were considered significant.

In the *Eptesicus fuscus* experiment I performed ANOVAs, 2-factor ANOVAs, t-tests and Kruskal-Wallis rank tests, to test whether sound- and behavioral parameters (i.a. distance of beginning of approach or end of buzz 1, flight speed, change rates of emission and echo SPL or PD and PI) differed significantly between different targets (single mealworm, mealworm cluster and reflector targets) and bats. Furthermore, a post hoc test (Tukey Kramer) specified which target differed significantly from the others.
As in the *Myotis* experiment, a regression analysis was performed to test whether echolocation parameters depended on target distance. Target distance was log transferred in case of SPL analysis to achieve a normal distribution of data. As before, p-values $< 0.05$ were considered significant.

## 4.3 Results

### 4.3.1 Experiments with *Myotis myotis*

#### 4.3.1.1 Ensonification of the mealworm and the reflector target

The two targets were ensonified at target distances between 0.4 and 1.2 m with 40 kHz sinusoidal signals (see chapter 4.2.6) that were produced with a calibrated loudspeaker. The signals had a duration of 1.5 ms, were repeated with delays of 30 ms and had an amplitude of 90 dB in a distance of 40 cm. For each distance, 80 data points were included in the analysis, if possible. The echo SPL values measured at different distances increased with 11.2 dB/hd ($y = -16.18\ln(x) + 28.856$) for the mealworm and 9.3 dB/hd ($y = -13.39\ln(x) + 27.337$) for the reflector (Fig. 9). The target strength (mean ± s.d.) in a distance of 1 m was -56.3 (± 2.6) dB for the mealworm and -49.2 (± 2.6) dB for the reflector, resulting in a mean TS-difference of 7 dB.

#### 4.3.1.2 Flight behavior

The flight behavior of the bats was quite similar regardless of whether they approached the mealworm or the reflector (Fig. 11, Table 1). The trained bats flew straight towards the targets (Fig. 3, Fig. 11 B). After starting from the grid, the flight height was initially slightly reduced and then increased again during the approach to the prey, resulting in a “u”-shaped trajectory (Fig. 11 A). The vertical width of the flight channel had a mean extent of 10 cm for flights towards the mealworm with a maximum of 13 cm (bat 3) and 9 cm mean for the flights towards the reflector with a maximum of 14 cm (bat 2). The horizontal width of the flight channel had a mean extent of 21 cm for flights towards the mealworm with a maximum of 24 cm (bat 4) and 20 cm mean for the flights towards the reflector with a maximum of 25 cm (bat 4) (Table 1). Thus, the horizontal widths of the flight channels were smaller than the wing span of about 35 – 45 cm.

**Table 1: Flight path characteristics** when catching a mealworm and the reflector. Values are mean ± s.d. N = 16 for each situation.

| | mealworm | reflector |
|---|---|---|
| min. flight height (m) | 1.17 ± 0.08 | 1.19 ± 0.09 |
| vertical width of flight channel (cm) | 10.3 ± 2.2 | 8.8 ± 3.9 |
| horizontal width of flight channel (cm) | 20.7 ± 2.8 | 20.2 ± 5.7 |
| max. speed (m/s) | 3.6 ± 0.1 | 3.6 ± 0.1 |
| speed when catching (m/s) | 1.5 ± 0.2 | 1.6 ± 0.5 |

Flight speed increased during the first third to half of the flight and decreased again (Fig. 11 C) after the maximum of 3.6 m/s in both situations (Table 1). A paired t-test confirmed no significant differences in maximum speed in both situations ($t = 0.14$, $df = 3$, $p = 0.9$). When catching the target, bats still had a mean speed of 1.5 m/s for the mealworm and 1.6 m/s for the reflector (Fig. 11 C, Table 1). Again, a paired t-test confirmed no significant differences in speed ($t = 0.41$, $df = 3$, $p = 0.71$).

Video recordings revealed that the mealworm was always caught by forming a pouch with the tail membrane. Sometimes the wings were also used to reach for the prey. In contrast, bats often tried to catch the much bigger reflector directly with the mouth. Sometimes, the wing was also used to catch this target.

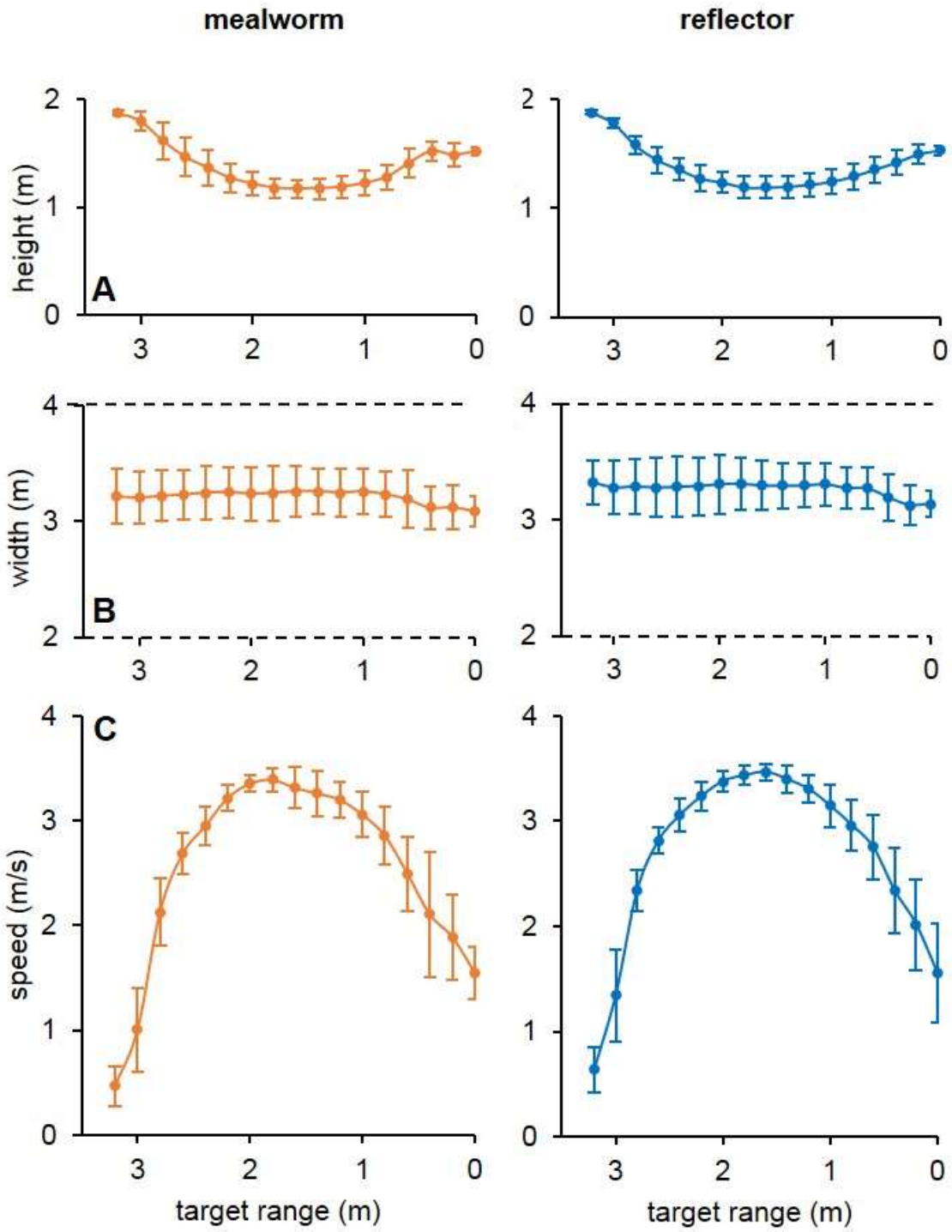



**Fig. 11: Average flight channel height (A), width (B), and flight speed (C)** (mean ± s.d.) of all bats at flights to the mealworm and to the reflector. N = 4 flights for each of the 4 bats. Flight behavior in distance bins of 20 cm.

#### 4.3.1.3 Echolocation behavior during the approach: emission pattern, sound duration and pulse interval

The echolocation behavior of the bats is described by the behavioral stages search and approach whereby the approach is subdivided in the two parts initial and terminal approach (Melcón et al., 2007, 2009). All these stages were observed in all bats while catching the two target types. All flights began with typical search behavior in which three bats emitted ungrouped echolocation calls with pulse intervals around 100 ms (Table 2). This most likely indicates the emission of one call per wingbeat. Bat 4, however, broke ranks and probably emitted two calls per wingbeat with a PI around 50 ms (Fig. 12 B, Table 2) in both the mealworm and the reflector trials. The Kruskal-Wallis test confirmed a significant difference between the bats (mealworm & reflector: $\chi^2 \geq 64.03$, df = 3, $p < 0.0001$; Steel-Dwass: bat 4 differed from all other bats, $p < 0.0001$). Mean pulse interval (± s.d.) during the search phase was for bat 1-3 97.6 (± 40.6) ms and for bat 4 52.7 (± 11.6) ms for trials with the mealworm and for bat 1-3 101.4 (± 38.7) ms and for bat 4 52 (± 8.4) ms for trials with the reflector.

**Table 2: Pulse duration (ms) and pulse interval (ms) (mean ± s.d.) during search phase** for each bat and both offered targets. N = 4 for each bat and condition.

| | Mealworm | | reflector | |
|---|---|---|---|---|
| | PD (ms) | PI (ms) | PD (ms) | PI (ms) |
| bat 1 | 1.4 ± 0.3 | 97.3 ± 42.2 | 1.5 ± 0.3 | 92.7 ± 43.9 |
| bat 2 | 1.6 ± 0.3 | 94.3 ± 38.4 | 1.8 ± 0.4 | 93.4 ± 38.1 |
| bat 3 | 1.5 ± 1.2 | 101.3 ± 41.3 | 1.9 ± 0.5 | 118.2 ± 54.4 |
| bat 4 | 2.2 ± 0.5 | 52.7 ± 11.6 | 2.2 ± 0.4 | 52 ± 8.4 |

Mean pulse duration was for bat 1-3 1.5 (± 0.3) ms and for bat 4 2.2 (± 0.5) ms for the mealworm trials and for bat 1-3 1.7 (± 0.4) ms and for bat 4 2.2 (± 0.4) ms for those trials with the reflector (Fig. 12 A, Table 2). Again, a Kruskal-Wallis test confirmed a significant difference between the bats (mealworm & reflector: $\chi^2 \geq 44.16$, df = 3, $p < 0.0001$). The post hoc Steel-Dwass test showed again the significant difference between bat 4 on the one hand and at least bats 1-2 on the other hand ($p \leq 0.0007$). Furthermore, a Wilcoxon test confirmed

that pulse intervals of the search phase didn't differ between the situations (Wilcoxon, $z = -0.21$, $n_{mw} = 137$, $n_{refl} = 124$, $p = 0.83$). However, pulse durations differed significant between both situations (Wilcoxon, $z = 2.40$, $n_{mw} = 145$, $n_{refl} = 129$, $p = 0.02$).

The emission level of search calls was fairly constant (compare Fig. 12 C), fluctuating around a mean value of 90.2 dB at the microphone with a standard deviation of 3.1 dB for mealworm targets and around a mean value of 87.8 dB at the microphone with a standard deviation of 5.1 dB for reflector targets. I found no significant difference between the approaches to different targets (paired t-test, $t = 2.02$, $df = 3$, $p = 0.14$). The averaged emission SPL at the microphone for bat 2-4 was 91.5 (± 2) dB and 86.4 (± 4.7) dB for bat 1 in trials with the mealworm and in reflector trials 90.2 (± 3.1) dB for bat 2-4 and 81 (± 4.9) dB for bat 1 (Table 3). The emission SPL differed significantly between the bats (Kruskal-Wallis test; mealworm & reflector: $\chi^2 \geq 56.11$, $df = 3$, $p < 0.0001$) with bat 2 and bat 4 having a more stable SPL in search calls while the others were more variable (Fig. 14, Table 3). In addition, the emission level of bat 1 was much lower than of the other three bats with an SPL between 86 and 81 dB (Table 3). The Steel-Dwass test confirmed this outstanding position of bat 1 ($p < 0.0001$).

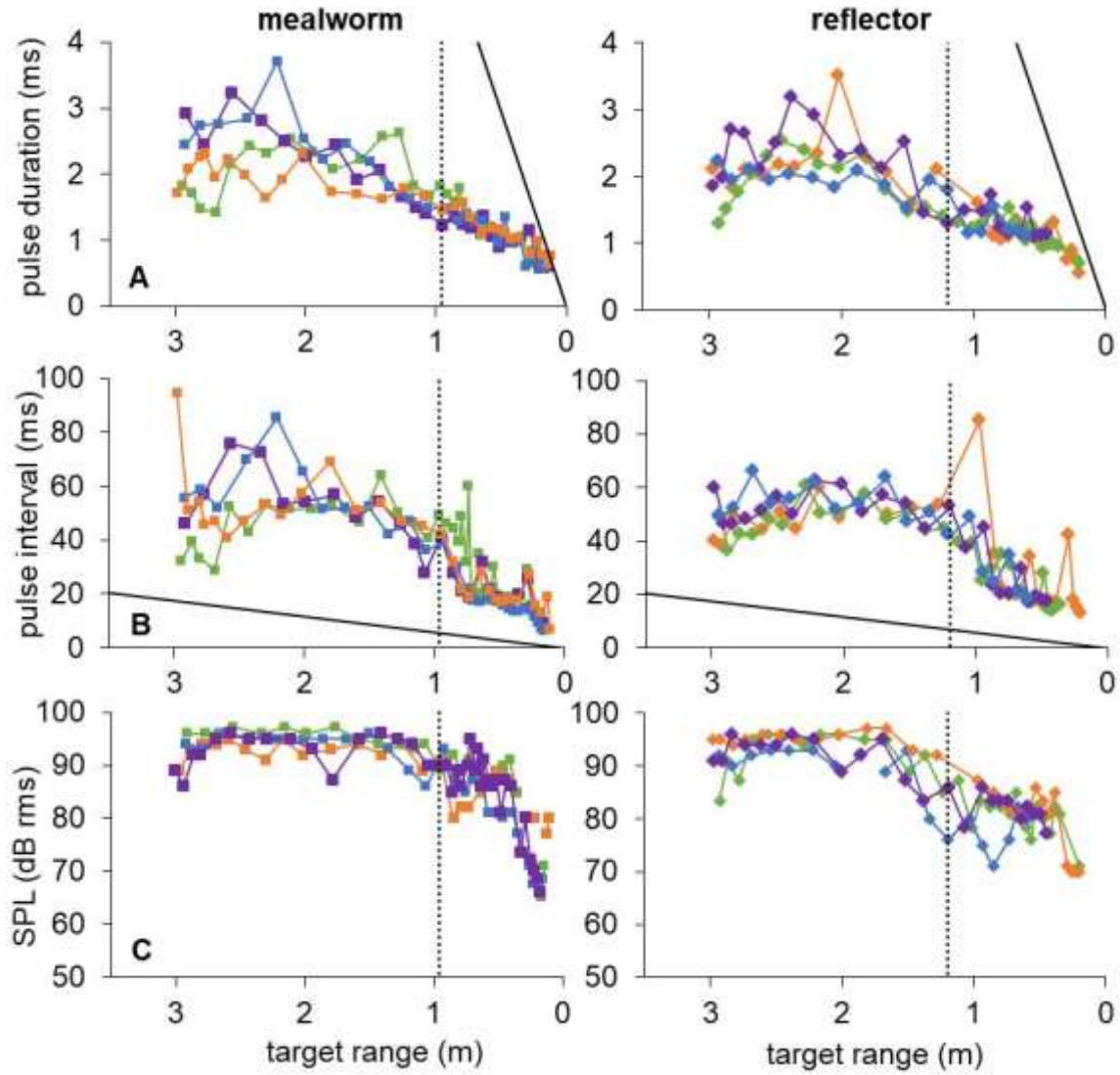



**Fig. 12: Echolocation behavior during 4 flights of bat 4** when approaching the mealworm or the reflector described by pulse duration (A), pulse interval (B) and emission SPL at the microphone (C) from search phase, initial approach and buzz 1. Buzz 2 signals are not included. The dashed black lines mark the averaged beginning of the approach. The solid black lines in A and B indicate the two-way travel times. Different colors represent different trials.

**Table 3: Bats' echolocation and target characteristics during *M. myotis* flights to (A) mealworm and (B) reflector targets.** Shown are the means (± s.d.) for each bat and the overall mean for each target: Measured emission SPL at the microphone during search phase (dB), reaction distance (m) for SPL reduction, emission SPL (dB) at the microphone at reaction distance of 1.03 (A) and 1.25 m (B), distance at the begin of buzz 1 (m), emission SPL at the microphone at the begin of buzz 1 (dB), distance at the end of buzz 1 (m), emission SPL at the microphone at the end of buzz 1 (dB), echo SPL at the end of buzz 1 (dB), absolute emission SPL reduction (dB) as the difference between SPL in search calls and SPL at the end of buzz 1, distance at the end of buzz 2 (m), emission SPL reduction (dB/hd), echo SPL increase (dB/hd), target strength (dB). N = 4 for each bat in each condition.

| **A** | emission SPL in search (dB) | reaction distance (m) | emission SPL at reaction distance (dB) | buzz 1 begin (m) | emission SPL at buzz 1 begin (dB) | buzz 1 end (m) | emission SPL at buzz 1 end (dB) | echo SPL at buzz 1 end (dB) | absolute emission SPL reduction (dB) | buzz 2 end (m) | emission SPL reduction (dB/hd) | echo SPL increase (dB/hd) | target strength (dB) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| bat 1 | 86.4 ±4.7 | 1.19 ±0.2 | 79.7 | 0.26 ±0.05 | 76.1 ±5.7 | 0.16 ±0.1 | 74.9 ±7.8 | 38.2 ±7.8 | 11.5 | 0.04 ±0.02 | 1.6 ±3.1 | 9.6 ±3.1 | - |
| bat 2 | 90.9 ±3.9 | 0.94 ±0.1 | 83.3 | 0.54 ±0.2 | 82.3 ±3.4 | 0.21 ±0.05 | 78.7 ±4.2 | 38.4 ±3.5 | 12.2 | 0.07 ±0.01 | 1.9 ±1.6 | 9.3 ±1.6 | - |
| bat 3 | 89.8 ±3.2 | 0.98 ±0.2 | 82.3 | 0.37 ±0.07 | 78.8 ±2.6 | 0.18 ±0.08 | 76.9 ±3.9 | 38.3 ±3.9 | 12.9 | 0.05 ±0.01 | 1.8 ±2.4 | 9.4 ±2.4 | - |
| bat 4 | 93.8 ±2.4 | 1.02 ±0.2 | 91.4 | 0.3 ±0.03 | 78 ±3.6 | 0.15 ±0.03 | 70.2 ±7 | 34.5 ±7 | 23.6 | 0.07 ±0.03 | 7.8 ±3.7 | 3.4 ±3.7 | - |
| **mean** | **90.2 ±3.1** | **1.03 ±0.1** | **84.2 ±5.1** | **0.37 ±0.1** | **78.8 ±2.6** | **0.17 ±0.03** | **75.2 ±3.7** | **37.4 ±1.9** | **15.1 ±5.7** | **0.06 ±0.02** | **3.3 ±3** | **7.9 ±3** | **-56.3 ±2.6** |

| **B** | emission SPL in search (dB) | reaction distance (m) | emission SPL at reaction distance (dB) | buzz 1 begin (m) | emission SPL at buzz 1 begin (dB) | buzz 1 end (m) | emission SPL at buzz 1 end (dB) | echo SPL at buzz 1 end (dB) | absolute emission SPL reduction (dB) | buzz 2 end (m) | emission SPL reduction (dB/hd) | echo SPL increase (dB/hd) | target strength (dB) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| bat 1 | 81 ±4.9 | 1.24 ±0.1 | 75.3 | 0.13 | 67.3 ±11.3 | 0.09 | 65.3 ±14.1 | 41.4 ±10.2 | 15.7 | 0.05 ±0.01 | 3 ±5.4 | 6.3 ±5.4 | - |
| bat 2 | 90.5 ±3.9 | 1.29 ±0.1 | 78.6 | 0.47 ±0.18 | 76.8 ±3.2 | 0.28 ±0.08 | 75.8 ±1.6 | 33.6 ±1.6 | 14.7 | 0.08 ±0.01 | 1.4 ±2.9 | 7.9 ±2.9 | - |
| bat 3 | 87 ±5.5 | 1.09 ±0.2 | 79.3 | 0.49 ±0.07 | 75.5 ±1.4 | 0.22 ±0.02 | 72 ±2.1 | 33.1 ±2.1 | 15 | 0.06 ±0.01 | 3 ±2.5 | 6.3 ±2.5 | - |
| bat 4 | 92.8 ±3.9 | 1.37 ±0.3 | 83.6 | 0.35 ±0.14 | 75.4 ±4.1 | 0.18 ±0.03 | 70.2 ±5.7 | 34 ±5.7 | 22.6 | 0.06 ±0.01 | 5.5 ±2.3 | 3.8 ±2.3 | - |
| **mean** | **87.8 ±5.1** | 1.25 ±0.1 | **79.2 ±3.4** | **0.36 ±0.2** | **73.8 ±4.3** | **0.19 ±0.08** | **70.8 ±4.4** | **35.5 ±3.9** | **17 ±3.8** | **0.06 ±0.01** | **3.2 ±1.7** | **6.1 ±1.7** | **-49.2 ± 2.6** |

The approach behavior was indicated by a distinct change in the signal parameters. During the approach pulses were often emitted in groups and the number of pulses per group increased when the bats were closing in on the target.

The initial part of the approach began with a distinct reduction in pulse interval, pulse duration, and emission SPL and contained only a few pulse groups: During the analyzed mealworm approaches the bats emitted a total of 163 initial approach calls with averaged 10.2 calls per approach varying from 7.3 to 12.5 calls between bats. During reflector approaches a total of 169 initial approach calls was emitted with averaged 10.6 calls per approach varying between bats from 8 to 12.8 calls. The terminal part of the approach consisted of the last group of consecutive pulses which were emitted before the bats contacted the target. This final group or buzz (Melcón et al., 2009) consisted always of two parts, buzz 1 and buzz 2. The pulse interval was continuously reduced during buzz 1. Buzz 2 was characterized by fairly constant minimal pulse intervals and low signal frequencies.

I defined the beginning of the approach phase as the beginning of the first call that had a shorter pulse duration and pulse interval than the mean values of the search calls minus one standard deviation.

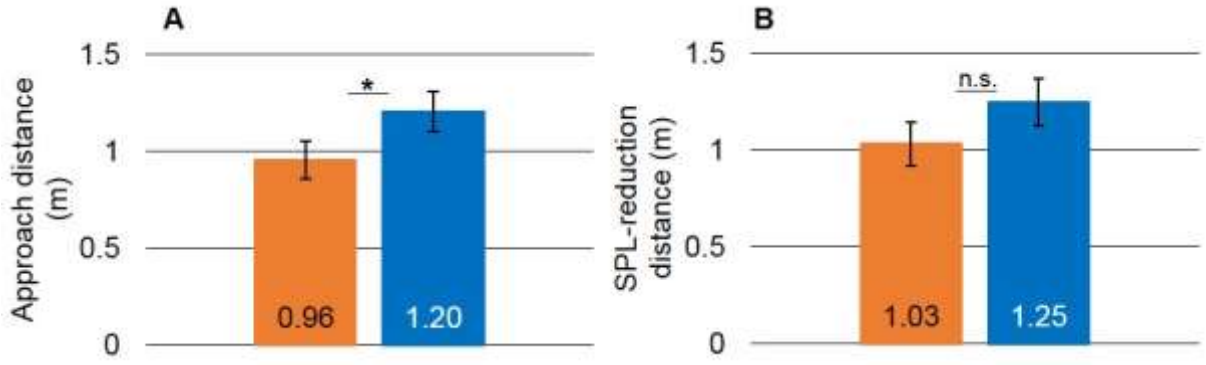



**Fig. 13: Averaged beginning of approach for the two target types defined by the reduction of PD and PI (A) or by SPL-reduction (B).** Targets are color-coded: orange = mealworm, blue = reflector. Error bars show standard deviation. N = 16 (4 flights from 4 bats at each target).

In experiments with the mealworm, the approach began at a mean target distance of 0.96 (± 0.1) m and in experiments with the reflector at 1.2 (± 0.1) m (Fig. 13 A). Thus, bats reacted in a significant larger distance to the loud target, the reflector, than to the weak target, the mealworm (2-factor ANOVA, $F(1,3) = 12.1$, $p = 0.04$). The target distance of the beginning of the approach did not differ between the bats (2-factor ANOVA, $F(3,3) = 1.05$, $p = 0.49$).

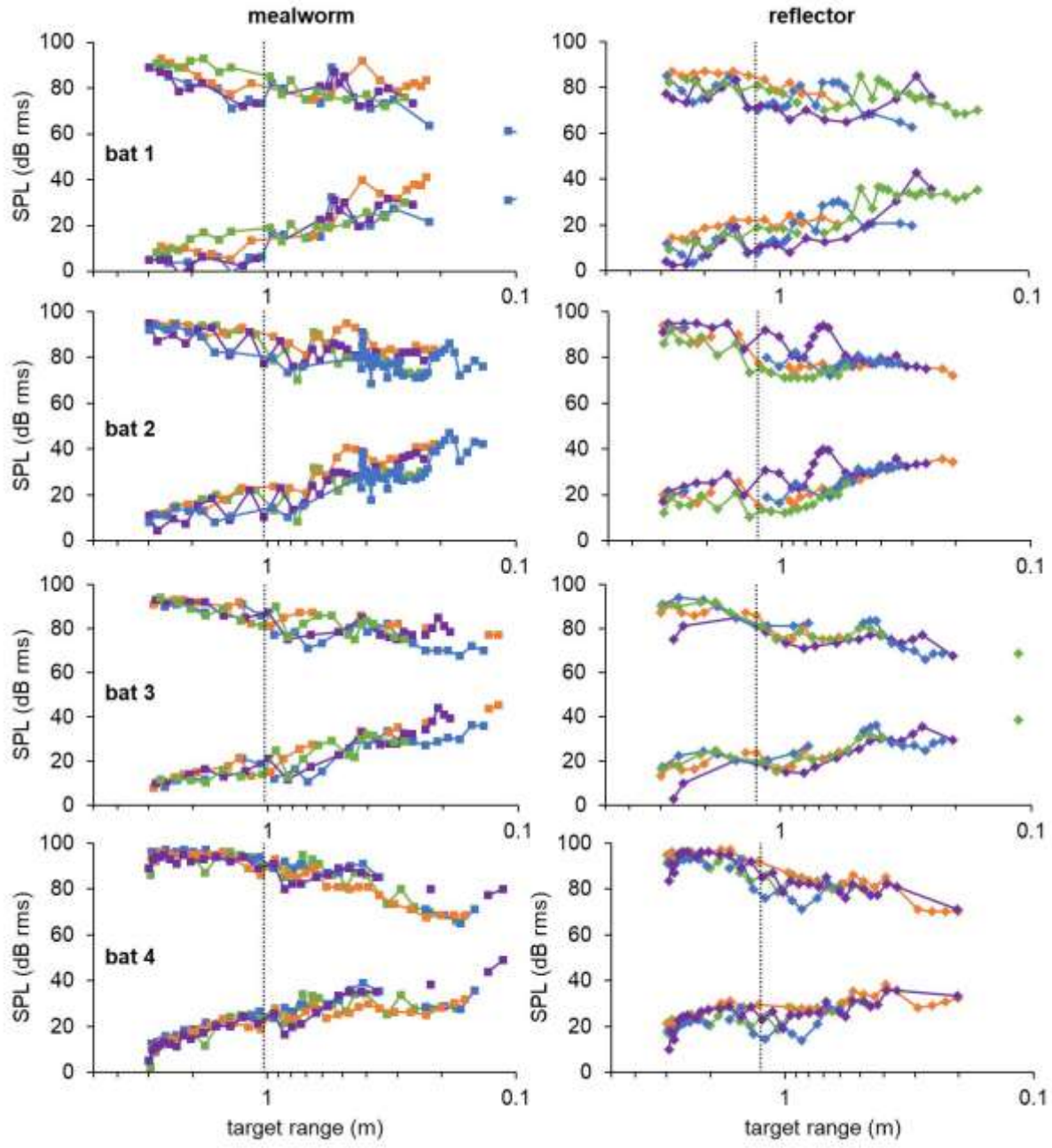



**Fig. 14: Measured emission- and calculated echo SPL** for all four bats in mealworm and reflector approaches. Upper data belong to the emission SPL and lower data to the echo SPL. The dashed lines mark the averaged beginning of the SPL reduction defined by a deviation of at least 2 * s.d. from the values of the search signals. Different colors represent different trials. N = 4 per bat and condition.

The terminal part of the approach, also called buzz, was always divided into buzz 1 and buzz 2. Buzz 1 contained 4 – 16 calls in mealworm trials and 5 – 10 calls in reflector trials with continually decreasing pulse interval (Fig. 12 B). The mean buzz 1 size was 9.2 ± 4.3 calls in trials with the mealworm and 5.6 ± 2.9 calls in trials with the reflector. Buzz 1 started at a mean target distance of 37 (± 12) cm in mealworm trials and 36 (± 16) cm reflector trials. To determine these mean target distances, I first averaged the position of the first buzz 1 call for each bat and then calculated the overall mean. The range over which bats were emitting buzz 1 had almost the same length for both targets (averaged 20 cm in mealworm and 17 cm in reflector trials), resulting in a mean target distance at the end of buzz 1 of 17 (± 3) cm in mealworm and 19 (± 8) cm in reflector trials (Table 3).

Buzz 2 was characterized by minimal and nearly constant pulse intervals (mealworm: 6.1 (± 0.9 ) ms; reflector: 6.2 (± 1.4) ms), minimal pulse duration (mealworm: 0.6 (± 0.2) ms; reflector: 0.7 (± 0.2) ms) and a lowered terminal frequency (mealworm: from 36.2 (± 5.1) kHz during buzz 1 down to 20.7 (± 5.2) kHz; reflector: from 35.2 (± 4) kHz down to 22.4 (± 7.1) kHz). Buzz 2 contained 3 – 10 calls (one outlier: 29) in mealworm trials and 3 – 14 calls in reflector trials. The mean buzz 2 size was 7.6 ± 3.6 calls in trials with the mealworm and 7.1 ± 2.9 calls in trials with the reflector.

During initial approach and buzz 1, the pulse interval was reduced (Fig. 12 B) with decreasing target distance (regression analysis, mealworm: $r^2$ (276) = 0.51; $p < 0.0001$; reflector: $r^2$ (210) = 0.36; $p < 0.0001$) and also the pulse duration (Fig. 12 A) was reduced (regression analysis, mealworm: $r^2$ (283) = 0.45; $p < 0.0001$; reflector: $r^2$ (222) = 0.20; $p < 0.0001$).

Throughout the initial approach phase and buzz 1, pulse intervals were higher than the two-way travel time of echolocation calls (Fig. 12 B), thus allowing the echoes to return without overlapping with subsequent outgoing calls. By using the pulse intervals at the beginning of the approach phase and at the end of buzz 1 and by subtracting the corresponding TWT to calculate the processing time, I get values of about 40 ms for the beginning of the approach phase and 7 ms for the end of buzz 1 in both situations. However, in some cases the TWT drops below the pulse duration (see Fig. 12 A mealworm case) at the very end of buzz 1 meaning that the returning echoes of buzz 1 in these cases overlap with the associated outgoing call.

#### 4.3.1.4 Echolocation behavior during the approach: emission SPL and calculated echo SPL

When approaching a target the four bats reduced the emission SPL with decreasing target distance (Fig. 14, Fig. 15, Table 3) (regression analysis, mealworm: $r^2$ (272) = 0.134, $p < 0.0001$; reflector: $r^2$ (214) = 0.110, $p < 0.0001$). By definition, the bats had responded to the target with an SPL reduction when their emission SPL deviated by at least 2 * standard deviations from the mean SPL of the search signals. Average reaction distances (calculated as the mean for each bat and finally the overall mean) were measured with 1.03 (± 0.11) m in mealworm and 1.25 (± 0.12) m in reflector trials (Table 3, Fig. 13 B). When approaching the loud reflector, bats reacted with reducing the emission SPL at a larger distance as they did for the weak mealworm (Fig. 13 B). Differences, however, were not statistically significant (2-factor ANOVA, F (1,3) = 7.19, p = 0.075). For each of the two different target types, the reaction distances did not differ between the bats (2-factor ANOVA, F (3,3) = 1.07, p = 0.48).

The distances measured with PI-reduction (0.96 m for mealworm and 1.2 m for reflector) or SPL-reduction (1.03 m for mealworm and 1.25 m for reflector) for the beginning of the approach differed slightly (Fig. 13). A statistical test, however, revealed no significant difference (paired t-test, t = 1.34, df = 7, p = 0.22).

By definition, at the onset of the response, the bats had already lowered the emission SPL by at least 2 * standard deviations from the mean SPL of the search signals. The emission SPL at the mean reaction distance was therefore distinctly lower than for search signals with an average of 6 dB for experiments with the mealworm and 8.6 dB for those with the reflector (Table 3).

In reacting bats, emission SPL reduction rates/hd differed between individuals (Table 3 A, B). Bat 4 showed the strongest reduction of emission SPL when approaching the mealworm and the reflector. I determined the mean SPL reduction rates by first calculating a mean for each bat and finally an overall mean. The mean SPL reduction rate for the mealworm trials was only slightly higher with 3.3 (± 3) dB/hd than for the reflector trials with a mean reduction of 3.2 (± 1.7) dB/hd (Table 3) and did not differ significantly (paired t-test, t = 0.09, df = 3, p = 0.9). The total SPL reduction (based on column 1 and 7, Table 3; the total SPL reduction

is determined as the mean value of the resulting difference) amounted 15 (± 5.7) dB for the mealworm trials and 17 (± 3.8) dB for the trials with the reflector and is therefore quite similar in both situations.

The total SPL reduction always resulted in an emission SPL of around 65 – almost 80 dB (Table 3) at the end of buzz 1, regardless of the target types offered (paired t-test, $t = 2.16$, $df = 3$, $p = 0.12$). In individual flights, the course of emission SPL is not continuously and strictly reduced during the approach, but rather an up and down (Fig. 12, Fig. 14).

Due to technical limits the Telemike allowed only direct echo SPL measurements above about 60 dB. The low TS of the mealworm (-56.3 dB) and reflector (-49.2 dB) target produced echoes with SPLs below 60 dB so that I could not measure echo SPLs and echo increase rates directly. However, in ensonification experiments an echo increase rate of 11.2 dB/hd was measured for the mealworm and 9.3 dB/hd for the reflector (Fig. 9). By using the available information on target distance, target strength and echo increase rates I calculated for each emitted signal the corresponding echo SPL (Fig. 14, Fig. 15).

From the difference between the echo increase rate of 11.2 dB/hd and the emission SPL reduction rate of 3.3 dB/hd, an echo increase rate at the microphone of 7.9 dB/hd in the mealworm experiment was calculated. Correspondingly, an increase rate at the microphone of 6.1 dB/hd was calculated for the reflector experiment from the difference of 9.3 dB/hd (echo increase rate) and 3.2 dB/hd (emission SPL reduction rate) (Table 3).

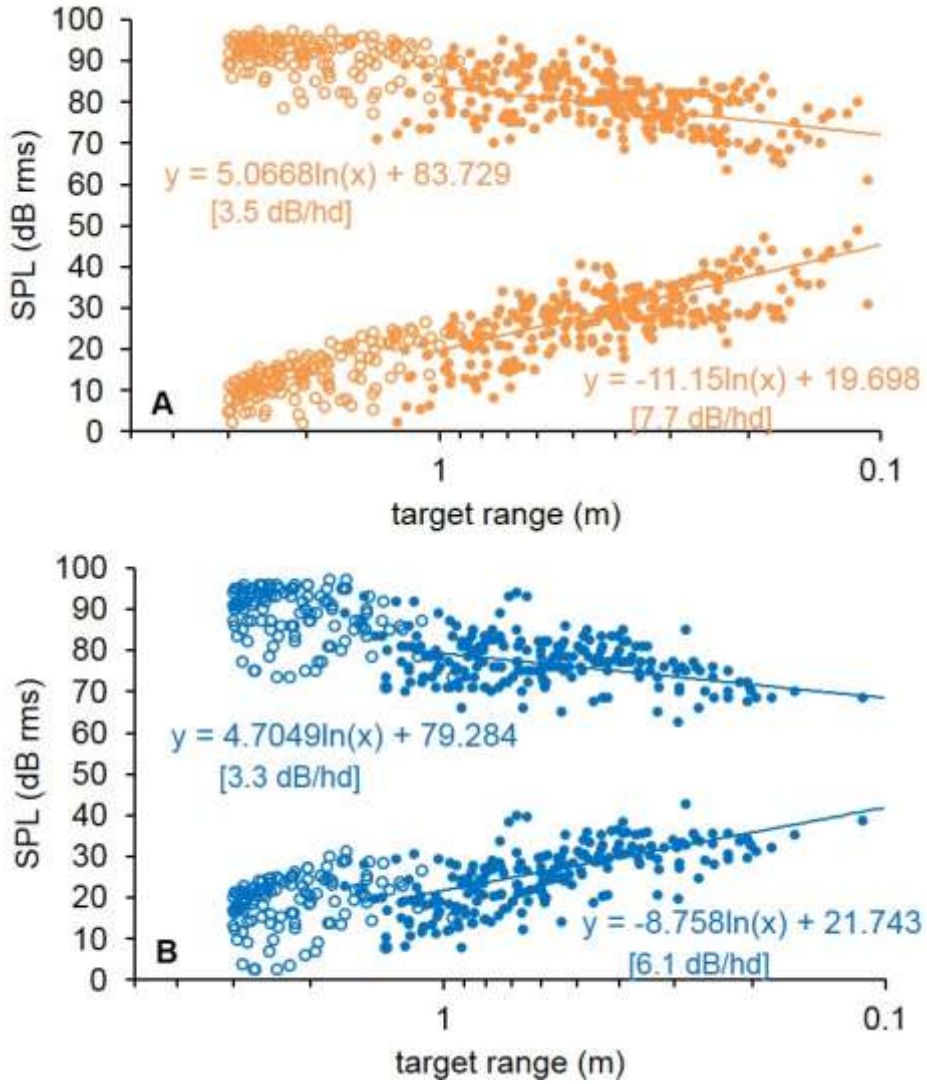



**Fig. 15: Measured emission SPL of *M. myotis* at the microphone and calculated echo SPL** for 4 flights of all four bats in mealworm (A) and reflector approaches (B) at a logarithmic range scale. Upper data belong to the emission SPL measured directly at the microphone. Lower data belong to the echo SPL that was calculated by taking into account the SL re 1 m, which is assumed to be 10 dB lower than the measured emission SPL at the microphone. Search signals are indicated by open circles and approach signals by closed circles. The slope of the model was calculated as mean of individual slopes of the 16 flights. Resulting SPL changes per halving distance are written below the formula.

At 1 m target distance the difference between the equation of the emission SPL reduction and of the echo increase rate corresponds to the target strength. Therefore, it is possible to determine the hypothetical echo SPL at the reaction distances of 1.03 m for the mealworm- and 1.25 m for the reflector situation. The mean reaction threshold is calculable by subtracting the TS from the averaged emission SPL at the reaction distance. With this approach a mean reaction threshold of approximately 20 dB was determined for both, the mealworm target and the reflector target.

I could determine the mean emission and echo SPL values at the distance of the beginning of approach as well as at the end of buzz 1 for both targets. The echo SPL in Fig. 15 is calculated based on the SL re 1 m which is about 10 dB lower than the measured SPL at the microphone. During mealworm approaches, the mean SPL at the beginning of approach was 84 dB. Simultaneously, the returning echo had an SPL of about 19 dB. At the target distance where buzz 1 ended, the emission SPL was about 75 dB and the echo SPL was about 37 dB (Table 3 A). I found nearly the same values during reflector approaches: here, the mean SPL at the beginning of approach was 80 dB. Simultaneously, the returning echo had an SPL of about 20 dB as well. At the end of buzz 1, the emission SPL was about 71 dB and the echo SPL was about 36 dB (Table 3 B). Thus, the relations between emission and echo SPL were nearly the same independent of target type and therefore target characteristics. The SPLs at the end of buzz 1 reached a difference in emission and echo SPL of about 38 dB for the mealworm and 35 dB for the reflector target.

### 4.3.2 Experiments with *Eptesicus fuscus*

#### 4.3.2.1 Ensonification of the three targets

All targets were ensonified with a sinusoidal signal at different distances and from various directions, just as described in chapters 4.2.6 and 4.3.1. The echo SPL values measured at different distances increased with a rate of 11.2 dB/hd for a single mealworm, 10.5 dB/hd for the cluster of four mealworms and 8.6 dB/hd for the reflector (Fig. 10, Table 4). The target strength (mean ± s.d.) in 1 m distance of a single mealworm was -56.3 (± 2.6) dB, of the cluster of mealworms -50.3 (± 2.6) dB and of the reflector -47.0 (± 3.4) dB (Table 4).

**Table 4: Target strength and echo SPL increase rate per halving of distance for a single mealworm, a cluster of four mealworms and the small reflector.** Target strength (dB) is given for a distance of 1 m.

| | single mealworm | cluster of 4 mealworms | reflector |
|---|---|---|---|
| TS (dB) | -56.3 (± 2.6) | -50.3 (± 2.6) | -47.0 (± 3.4) |
| increase rate (dB/hd) | 11.2 | 10.5 | 8.6 |

#### 4.3.2.2 Flight behavior

The aerial-hawking big brown bats that performed this experiment were not trained to use a distinct flight path prior to the recordings. Thus, I could observe various flight paths that are quite typical for these bats when foraging in a flight room (Fig. 16): Sometimes the bats were first circling around the target in the flight room once or twice before catching it. In other trials bats were approaching the target in slightly or stronger curved paths from the wall or the bats approached the targets in nearly straight paths. Mostly, however, bats approached the target in slight curves or semi-circumnavigated it before catch. No bat ever showed a preference concerning direction of approach or flight path. With a target height of 1.7 m flight height always fluctuated between a minimum of 0.95 m (R17 in an approach to the cluster of mealworms) and a maximum of 2.32 m (R17 in a single mealworm approach). Sometimes bats approached the target from above while in other trials they approached it from below (Fig. 16 A). I could not find any striking differences in flight behavior between the different

targets (Table 5). Dependent on the bats' flight path, flight speed varied with slower parts when flying a curve and faster parts when flying more or less straight forward.

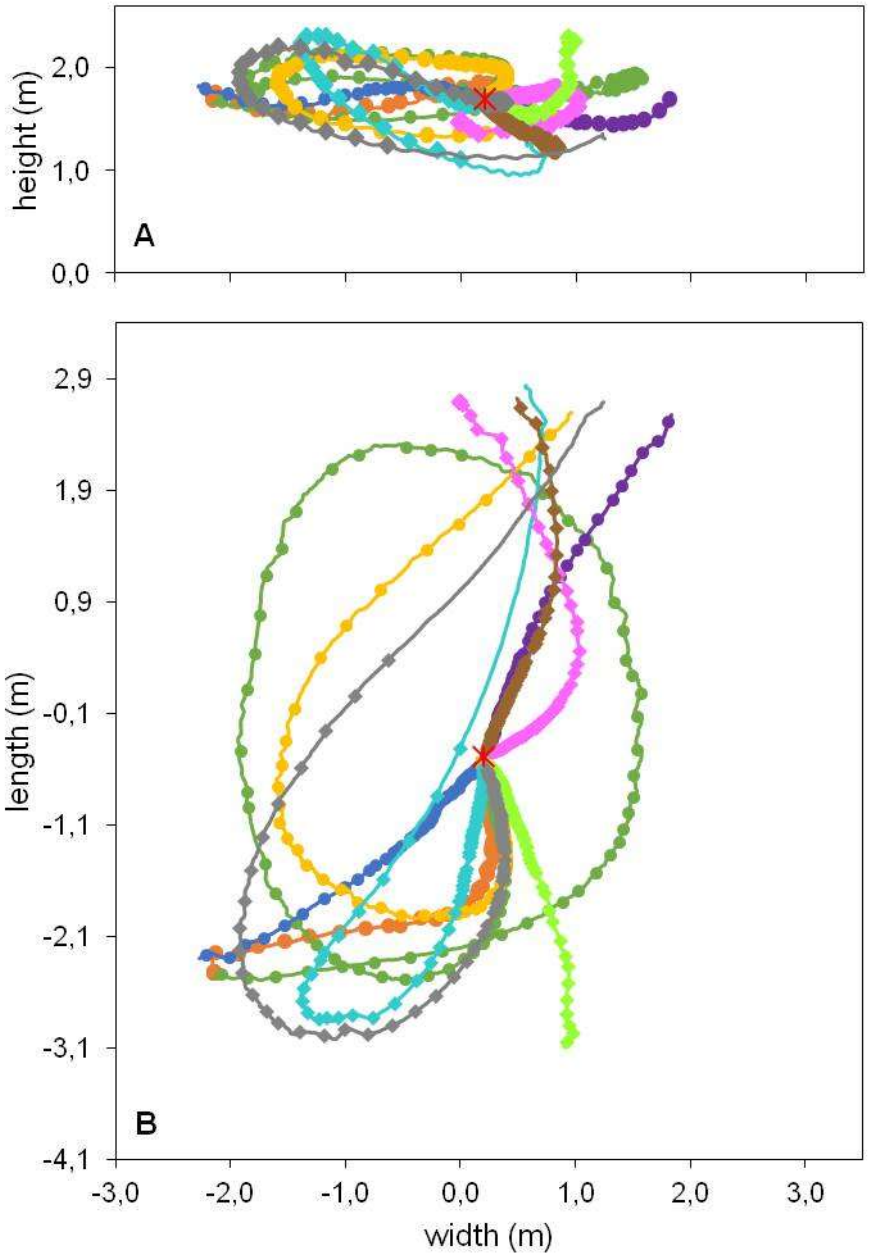



**Fig. 16: Flight behavior of both *E. fuscus* when approaching the cluster of four mealworms in side view (A) and top view (B).** Bats are symbol-coded: sphere = R19, rhomb = R17; the target is marked with a red asterisk. N = 10.

During the approaches to all three targets averaged maximum flight speed did not differ significantly (Table 5) from each other (ANOVA, $F_{(2,27)} = 0.55$, $p = 0.59$). Maximum flight speed varied between 4.1 m/s when approaching the cluster of four mealworms and 3.8 m/s during the approach of the reflector. Flight speed was lowest at the moment of capture. In the single mealworm condition *E. fuscus* had an averaged flight speed of 1.9 (± 1.1) m/s, in the mealworm cluster condition of 2.5 (± 0.7) m/s and in the reflector condition of 2.1 (± 0.7) m/s (Table 5). Flight speeds did neither differ significantly with the targets nor with the two individuals (2-factor ANOVA, $F_{(5,24)} = 1.08$, $p = 0.40$).

**Table 5: Flight path characteristics** when catching a single mealworm, the cluster of four mealworms and the reflector. Values are mean ± s.d. N = 10 for each situation.

| | single mealworm | cluster of 4 mealworms | reflector |
|---|---|---|---|
| min. flight height (m) | 1.42 ± 0.13 | 1.36 ± 0.23 | 1.39 ± 0.14 |
| max. flight height (m) | 1.93 ± 0.20 | 1.92 ± 0.26 | 1.90 ± 0.26 |
| max. speed (m/s) | 4 ± 0.6 | 4.1 ± 0.4 | 3.8 ± 0.3 |
| average speed when catching (m/s) | 1.9 ± 1.1 | 2.5 ± 0.7 | 2.1 ± 0.7 |

When approaching a target *E. fuscus* reduced its flight speed only during the last approximately 50 cm and thus only about 80 – 90 ms before catching, dependent on actual flight speed. Fig. 17 A shows the flight speed of an exemplary flight of *E. fuscus* (R17) when it approached the cluster consisting of four mealworms in straight flight. Up to the last approximately 50 cm before the catch, flight speed was quite constant with approximately 3.5 m/s. Only then the bat decelerated and thus reduced flight speed to just below 2 m/s when it caught the target. Flight speed varied more over the distance as the bats performed curves while flying towards the targets. Fig. 17 B illustrates this issue with bat R19 flying towards the reflector. Please note the low flight speed of less than 1 m/s at a distance of still 2 m which first increases before it again decreases at the end to just below 2 m/s.

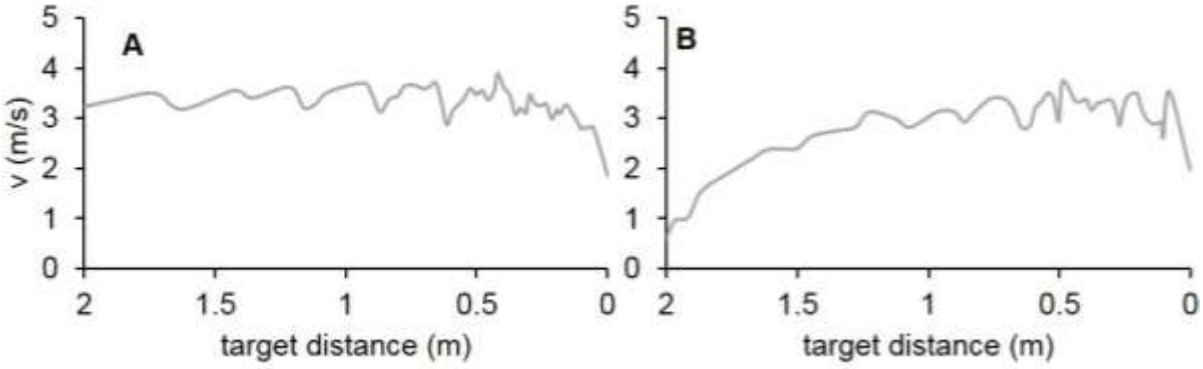



**Fig. 17*:* Exemplary flight speed of *E. fuscus* R17 while approaching the cluster consisting of four mealworms in straight forward flight (A) and of *E. fuscus* R19 while approaching the reflector performing a large curve (B).** Flight speed is shown only for the last two meters of the flight. (A) corresponds to the brown curve in Fig. 16 where the bat was flying more or less straight ahead while (B) corresponds to another target (without figure of flight path) to which the bat approached in a curve.

The way bats caught the targets did not differ between the objects. Targets were always captured by forming the typical pouch with the tail membrane (Fig. 18 A-C: pictures 4). During the first part of approach, the bat was still in normal flight mode. The tail membrane was then opened by a downward movement about 15 cm in front of the target (Fig. 18 A: pictures 2, 3). Here, the bat was already at the end of buzz 2 in echolocation behavior. Immediately before reaching the target, the tail membrane was lifted upwards. In echolocation behavior, the bat was halfway through the pause interval between the end of buzz 2 and the capture. The bat bended its head into the pouch and grabbed the mealworm out of the tail membrane. The wings were not involved in handling the prey. Then the bat righted itself again and continued to fly, again echolocating in search mode.

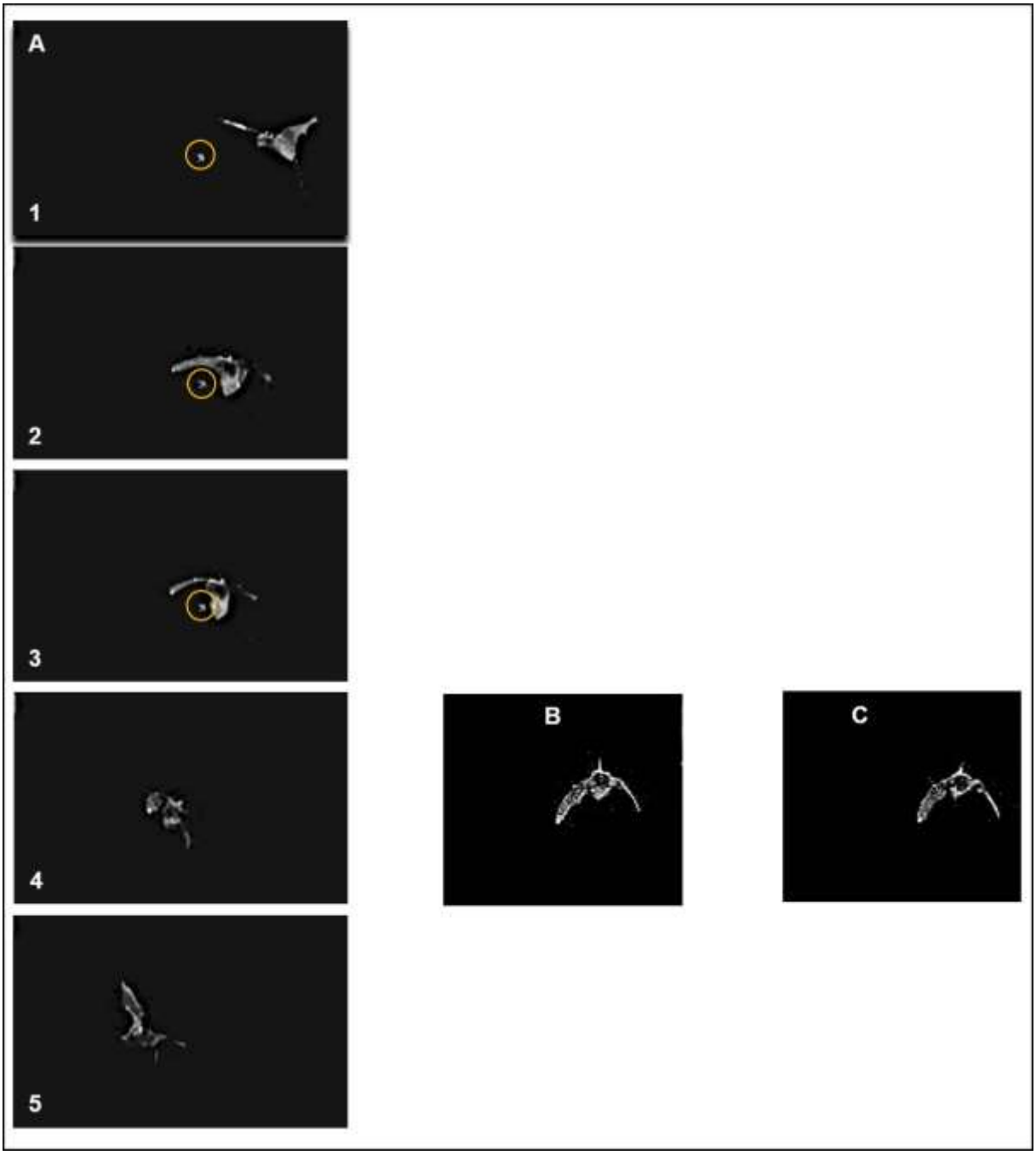



**Fig. 18: typical catching behavior of *E. fuscus* (here from R19) when catching a cluster of 4 mealworms (A), an artificial reflector (B) and a single mealworm (C).** The mealworm cluster is encircled in orange. In picture 1 the bat is still in typical flight movement. In picture 2 the bat starts to form a pouch with its tail membrane. Picture 3 shows the bat immediately before the catch with a broad and open tail membrane. Picture 4 shows the moment of the final catch – here the bat closes its pouch by forming a ball with its whole body and picking the prey up with its mouth. Only the wings that are not used for the catching process are sticking out. Picture 5 finally shows the bat flying shortly after the catch. In the cases of reflector catch (B) and single mealworm catch (C) only the "ball stadium" of the bat is shown to emphasize the same catching strategy for all targets, even for the reflector.

#### 4.3.2.3 Echolocation behavior during the approach: emission pattern, sound duration and pulse interval

During all foraging sessions of *E. fuscus* with all three different targets I could identify the typical stages search and approach. The approach was again subdivided in initial and terminal approach (Melcón et al., 2007, 2009). All flights started with typical search behavior which differed from following approach behavior. Typical pulse intervals for search phase ranged from 50 to 100 ms. During the search phase R19 emitted echolocation calls with mean pulse intervals between 66 ms and 75 ms and R17 with mean pulse intervals between 52 ms and 70 ms (Table 6). R19 had the largest PIs in the search phase of the reflector situation, while R17 has the largest PIs in the search phase of the single mealworm situation. Pulse intervals did not differ significantly between the situations (Kruskal-Wallis, $\chi^2 = 1.93$, df = 2, p = 0.38) but slightly between bats (Kruskal-Wallis, $\chi^2 = 8.26$, df = 1, p = 0.04). Mean pulse interval for both bats was 67.9 (± 2.3) ms for single mealworm trials, 64.8 (± 3.8) ms for mealworm cluster trials and 63.2 (± 16.1) ms for reflector trials.

Pulse duration was similar in both bats and measured between 2.3 ms and 2.6 ms (Table 6). Pulse durations of reflector trials differed significantly from those with single mealworm and mealworm cluster (2-factor ANOVA, F (2,5) = 4.84, p = 0.008; post-hoc Tukey-Kramer HSD). Pulse duration did not differ significantly between the bats (2-factor ANOVA, F (1,5) = 1.85, p = 0.17).

**Table 6: Pulse duration (ms) and pulse interval (ms) (mean ± s.d.) during search phase** for both bats and all offered targets. N = 4 for each bat and condition.

| | single mealworm | | cluster of 4 mealworms | | reflector | |
|---|---|---|---|---|---|---|
| | PD (ms) | PI (ms) | PD (ms) | PI (ms) | PD (ms) | PI (ms) |
| R19 | 2.3 ± 0.7 | 66.3 ± 29.5 | 2.4 ± 0.7 | 67.5 ± 23 | 2.6 ± 0.5 | 74.6 ± 16.8 |
| R17 | 2.5 ± 0.6 | 69.5 ± 22.6 | 2.6 ± 0.5 | 62.1 ± 16.4 | 2.6 ± 0.5 | 51.8 ± 17.7 |

Approach behavior was indicated by a distinct change in the signal parameters pulse interval, pulse duration and grouping of echolocation calls. The initial part of the approach began with a reduction of PI and PD and contained only a few pulse groups: During the approach to a single mealworm the bats emitted 10.6 (± 7) calls on average per approach. During the initial approach to the mealworm cluster, the mean number of calls was 9.8 (± 4.3) and during approach to the reflector the mean number of calls was 10.2 (± 3). A statistical test showed no significant differences in number of calls during the initial approach phase between the approaches to the three different targets (2-factor ANOVA, $F(2,29) = 0.07$, $p = 0.93$). On average, R17 always emitted significantly less calls (8 ± 0.7) during initial approach than R19 (12.4 ± 1.5) (2-factor ANOVA, $F(1,29) = 6.73$, $p = 0.02$).

The terminal part of the approach consisted of the last group of consecutive pulses which were emitted before the bats contacted the target. This final group consisted always of two parts, buzz 1 and buzz 2. While pulse intervals were still reduced during buzz 1, buzz 2 was characterized by rather constant minimal pulse intervals and lower terminal frequencies. Buzz 1 consisted of 10.1 (± 5) calls during approaches to a single mealworm, 13.4 (± 4.6) calls during approaches to the mealworm cluster and 14.1 (± 5.1) calls during approaches to the reflector. A statistical test confirmed that neither the bats nor the targets had a significant impact on call number of buzz 1 (2-factor ANOVA, $F_{bats}(1,29) = 0.22$, $F_{targets}(2,29) = 2.12$, $p \geq 0.14$). In buzz 2, bat and target had also no significant effect on call number (2-factor ANOVA, $F_{bats}(1,29) = 0.16$, $F_{targets}(2,29) = 0.31$, $p \geq 0.69$. The average number of emitted buzz 2 calls obviously did not differ with different targets: For single mealworm approaches I found a mean of 21 (± 5.4) calls, for approaches to the mealworm cluster 22.6 (± 4.4) calls and for the reflector approaches 21.1 (± 4.6) calls.

Approach started at a mean target distance of 1.32 (± 0.21) m in trials with the single mealworm, at a mean target distance of 1.61 (± 0.11) m in trials with the cluster of mealworms and at 1.73 (± 0.26) m in trials with the reflector (Fig. 19). A statistical analysis confirmed a significant difference in begin of approach between the single mealworm target and both, the mealworm cluster and reflector target (2-factor ANOVA, $F(2,5) = 9.94$, $p = 0.0007$, Tukey-Kramer, $p<0,05$). Distance to the target at the beginning of approach did not differ between the bats (2-factor ANOVA, $F(1,5) = 0.10$, $p = 0.76$).

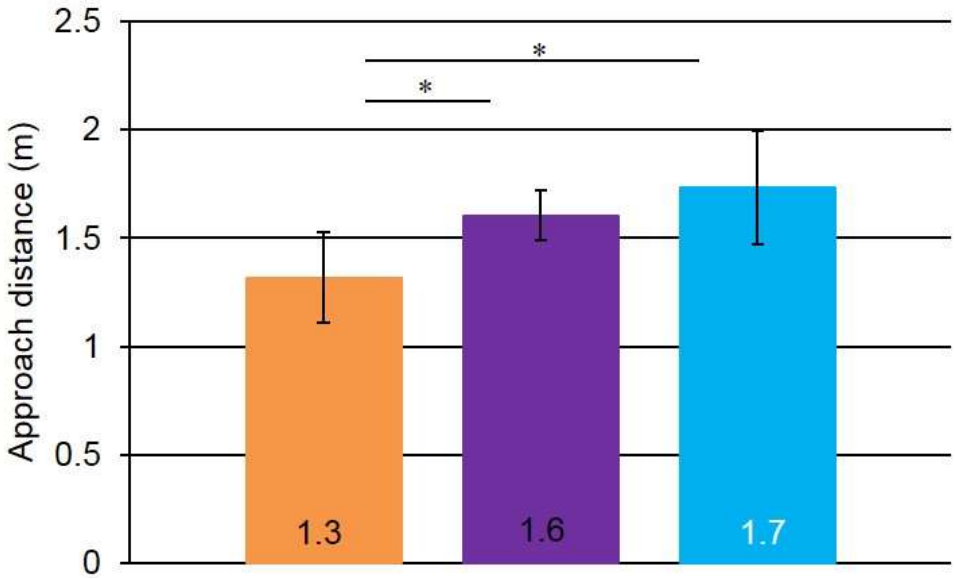



**Fig. 19: Averaged beginning of approach (m) for different target types defined by the reduction of PD and PI.** Targets are color-coded: orange = mealworm, violet = mealworm cluster, blue = reflector. Error bars show standard deviation. N = 10 for each target (5 flights from 2 bats at each target).

**Table 7: Bats' echolocation and target characteristics during *E. fuscus* flights to (A) single mealworm, (B) mealworm cluster and (C) reflector targets.** Shown are the means (± s.d.) for each bat and the calculated mean for each target: distance of begin of approach (m), hypothetical emission SPL (dB) at the microphone at begin of approach (values are those of lines of best fit), distance at the begin of buzz 1 (m), emission SPL (dB) at the microphone at the begin of buzz 1 (values are those of lines of best fit), distance at the end of buzz 1 (m), emission SPL (dB) at the microphone at the end of buzz 1 (values are those of lines of best fit), echo SPL at the end of buzz 1 (values are those of lines of fest fit), absolute emission SPL reduction (dB) (from the beginning of approach to the end of buzz 1, where the mean value is determined from line of best fit considering all bats and flights), emission SPL reduction (dB/hd) (where the mean value is determined from line of best fit considering all bats and flights), echo SPL increase (dB/hd) (where the mean value is determined from line of best fit considering all bats and flights), target strength (dB). N = 5 for each bat in each condition.

| **A** | approach begin (m) | emission SPL at approach begin (dB) | buzz 1 begin (m) | emission SPL at buzz 1 begin (dB) | buzz 1 end (m) | emission SPL at buzz 1 end (dB) | echo SPL at buzz 1 end (dB) | absolute emission SPL reduction (dB) | emission SPL reduction (dB/hd) | echo SPL increase (dB/hd) | target strength (dB) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| bat 1 | 1.3 ±0.25 | 104.3 | 0.66 ±0.2 | 95.8 | 0.4 ±0.1 | 90.3 | 36.4 | 14 | 9.3 | 1.9 | - |
| bat 2 | 1.33 ±0.19 | 108.5 | 0.74 ±0.4 | 96.3 | 0.57 ±0.3 | 88.4 | 34.6 | 20.1 | 13.3 | -2.1 | - |
| **mean** | **1.32 ±0.22** | **106.4 ±3** | **0.7 ±0.3** | **96.1 ±0.4** | **0.5 ±0.1** | **89.4 ±1.3** | **35.5 ±1.3** | **17 ±4.3** | **11.3 ±2.8** | **-0.1 ±2.8** | **-56.3 ±2.6** |

| **B** | approach begin (m) | emission SPL at approach begin (dB) | buzz 1 begin (m) | emission SPL at buzz 1 begin (dB) | buzz 1 end (m) | emission SPL at buzz 1 end (dB) | echo SPL at buzz 1 end (dB) | absolute emission SPL reduction (dB) | emission SPL reduction (dB/hd) | echo SPL increase (dB/hd) | target strength (dB) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| bat 1 | 1.63 ±0.13 | 106.8 | 0.85 ±0.1 | 96.7 | 0.51 ±0.1 | 87.5 | 37.5 | 19.2 | 11.6 | -1.1 | - |
| bat 2 | 1.58 ±0.10 | 104.9 | 0.92 ±0.1 | 96.8 | 0.56 ±0.1 | 89.4 | 39.3 | 15.5 | 9.4 | 1.1 | - |
| **mean** | **1.61 ±0.15** | **105.8 ±1.3** | **0.9 ±0.1** | **96.8 ±0.1** | **0.5 ±0.1** | **88.5 ±1.3** | **38.4 ±1.3** | **17.4 ±2.6** | **10.5 ±1.6** | **0 ±1.6** | **-50.3 ±2.6** |

| **C** | approach begin (m) | emission SPL at approach begin (dB) | buzz 1 begin (m) | emission SPL at buzz 1 begin (dB) | buzz 1 end (m) | emission SPL at buzz 1 end (dB) | echo SPL at buzz 1 end (SPL) | absolute emission SPL reduction (dB) | emission SPL reduction (dB/hd) | echo SPL increase (dB/hd) | target strength (dB) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| bat 1 | 1.68 ±0.19 | 103.1 | 0.84 ±0.2 | 98.3 | 0.5 ±0.1 | 94.2 | 45.4 | 8.9 | 5.1 | 3.5 | - |
| bat 2 | 1.79 ±0.33 | 108.6 | 0.97 ±0.2 | 101.2 | 0.58 ±0.2 | 94.9 | 46.1 | 13.7 | 7.8 | 0.7 | - |
| **mean** | **1.73 ±0.26** | **105.8 ±3.9** | **0.91 ±0.2** | **99.8 ±2.1** | **0.5 ±0.1** | **94.6 ±0.5** | **45.7 ±0.5** | **11.3 ±3.4** | **6.5 ±2** | **2.1 ±2** | **-47 ±3.4** |

Buzz 1 started at a mean target distance of 69.9 (± 28.0) cm in single mealworm, at 88.1 (± 11.5) cm in mealworm cluster and at 90.5 (± 22.3) cm in reflector trials. To determine these mean target ranges I first averaged the distance of the first buzz 1 call for each bat and then calculated the overall mean. Despite of a different target distance for the beginning of buzz 1, the target distance at the end of buzz 1 was quite similar for all targets with 48 (± 12) cm in mealworm, 53 (± 10) cm in mealworm cluster and 54 (± 15) cm in reflector trials (Table 7). This result was confirmed by a statistical test (ANOVA, $F(2,27) = 0.32$, $p = 0.73$).

Buzz 2 was characterized by minimal and constant pulse intervals of 5.9 ± 0.2 ms (total mean ± standard deviation). Pulse duration was also shortest in buzz 2 with 0.99 ± 0.06 ms and terminal frequency was reduced, though only slightly, as usual for the transition from buzz 1 to buzz 2 (from 22.4 (± 0.2) kHz during buzz 1 down to 19.1 (± 0.03) kHz during buzz 2). Buzz 2 contained 12 – 27 calls in the single mealworm trials, 15 – 27 calls in mealworm cluster trials and 13 – 27 calls in reflector trials. The mean buzz 2 size was nearly the same for all targets with 21 (± 5.4) calls for single mealworm approaches, 22.6 (± 4.4) for mealworm cluster approaches and 21.1 (± 4.6) calls for reflector approaches. A statistical test carried out confirmed this result and found no statistical significance (Kruskal-Wallis test, $\chi^2 = 0.47$, $df = 2$, $p = 0.79$).

During initial approach and buzz 1 pulse duration (Fig. 20 A) and pulse interval (Fig. 20 B) were reduced with decreasing target distance (PD: regression analysis, single mealworm: $r^2(205) = 0.32$, $p < 0.0001$; mealworm cluster: $r^2(230) = 0.42$, $p < 0.0001$; reflector: $r^2(240) = 0.41$, $p < 0.0001$ and PI: regression analysis, single mealworm: $r^2(202) = 0.42$, $p < 0.0001$; mealworm cluster: $r^2(203) = 0.64$, $p < 0.0001$; reflector: $r^2(240) = 0.62$, $p < 0.0001$). Simultaneously, *E. fuscus* arranged their echolocation calls in groups. Thereby the number of calls within a group was increased with decreasing distance to the target (see Fig. 20 B).

During initial approach and buzz 1 the two-way travel time (TWT) was always higher than pulse duration (Fig. 20 A). Thus, the returning echoes never overlapped with the associated outgoing calls. Throughout the initial approach phase and buzz 1, pulse intervals were higher than the TWT of echolocation calls (Fig. 20 B) and thus allowed the echoes to also return

without any overlap with subsequent outgoing calls. Taking into account the pulse interval and TWT, *E. fuscus* has a processing time of 51 ms at the beginning of the approach phase and of 4 ms towards the end of buzz 1 when approaching a single mealworm. During mealworm cluster and reflector approaches processing time at the end of buzz 1 is also at the minimum of scantily 4 ms but at the begin of approach it is 10 ms less than in single mealworm approaches, namely just above 40 ms.

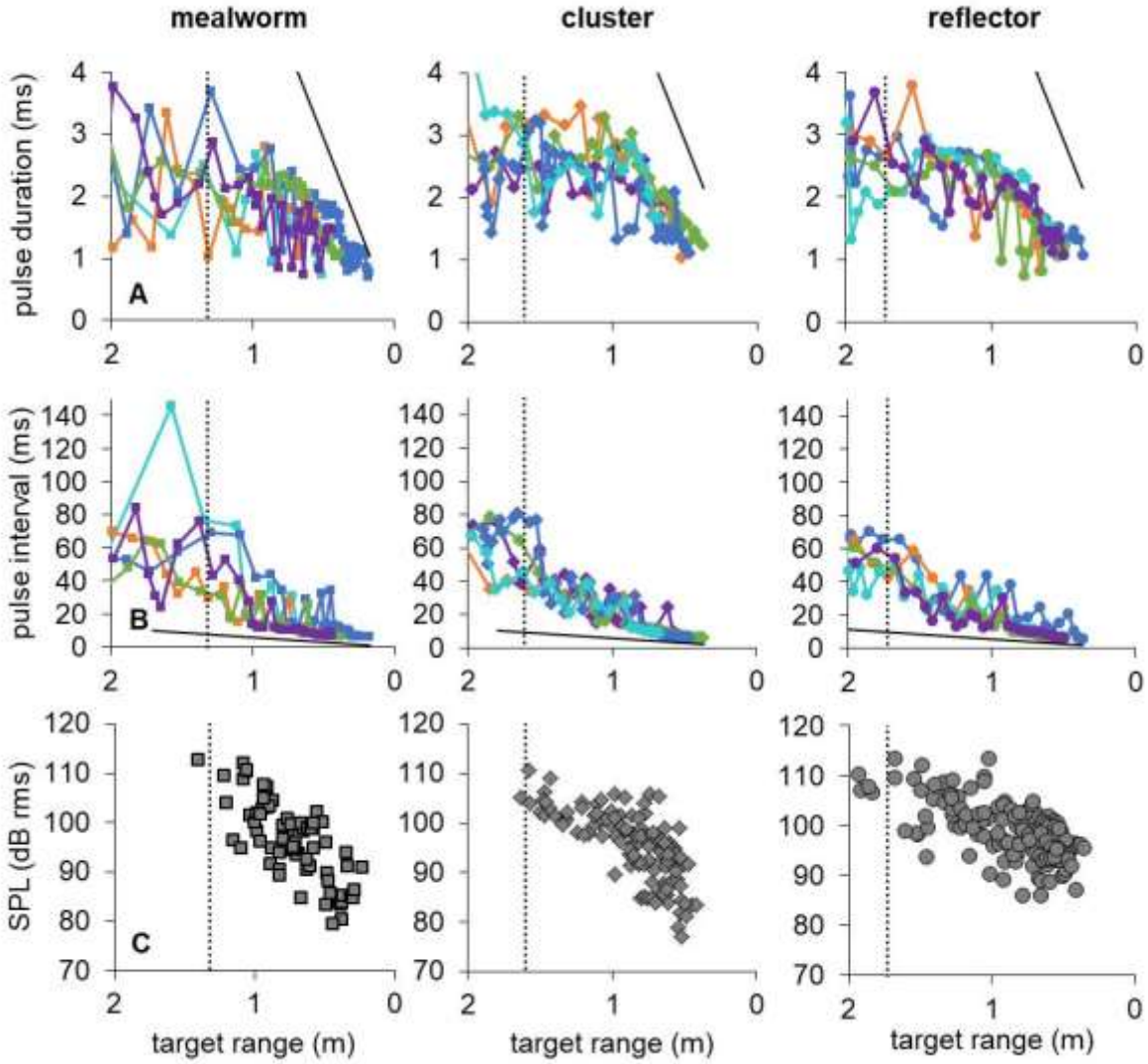



**Fig. 20: Echolocation behavior of *E. fuscus*** during 5 flights of R19 when approaching the single mealworm, the mealworm cluster or the reflector described by pulse duration (A), pulse interval (B). (C) shows the emission SPL at the microphone calculated from the recorded echoes for the entirety of the data (5 flights of two animals each) from initial approach and buzz 1. Buzz 2 signals are not included. The dashed black lines mark the averaged beginning of the approach. The solid black lines in A and B indicate the two-way travel times. The different trials are color-coded.

#### 4.3.2.4. Echolocation behavior during the approach: echo SPL and calculated emission SPL

As described above, in the study with *E. fuscus* I was most interested in recording echoes that returned from the target of interest. Therefore, I used a quite sensitive recording system and therewith accepted that the outgoing echolocation calls were in the overload. Thus, the emission SPLs were calculated from the received echo SPLs. Since the calibration of the absolute SPLs was not applicable in this experiment due to methodical problems (see methods) the absolute values were recalibrated by setting the value of the regression of the emission SPL to 106 dB at the beginning of the approach, which corresponds to the emission SPL measured during search flight in small rooms (Koblitz et al., 2010; Weiss, 2021). Absolute values are thus most plausible values. Relative measures such as the reduction of emission level or the increase of echo SPLs and thus also the relation of emission SPL to echo SPL in Fig. 21 are not affected by this recalibration.

Echoes from the target were recorded up to a maximum target distance of 1.4 m for the single mealworm, 1.6 m for the cluster of mealworms and 1.9 m for the reflector, respectively. These distances correspond closely to the beginning of the approach determined by changes in pulse interval and pulse duration. When approaching the target of interest in all three conditions both *E. fuscus* reduced the emission SPL (Fig. 20 C, Fig. 21) with decreasing target distance (regression analysis, single mealworm: $r^2$ (72) = 0.58, $p < 0.0001$; mealworm cluster: $r^2$ (115) = 0.51, $p < 0.0001$); reflector: $r^2$ (141) = 0.43, $p < 0.0001$). Almost similar strong reactions in SPL reduction were found in the bats approaching the single mealworm (Fig. 20 C, Fig. 21 A) and the mealworm cluster (Fig. 20 C, Fig. 21 B). In single mealworm approaches, bats reduced emission SPL with 11.3 dB/hd. The corresponding echo SPL was kept almost constant (equations in Fig. 21 A) at approximately 36 dB. When approaching the cluster of mealworms, the echo SPL did neither change substantially with the approach to the target. The emission SPL was reduced with 10.5 dB/hd resulting in an almost constant echo SPL at the bats' ear of about 38 dB. When approaching the reflector, emission SPL was reduced with only 6.5 dB/hd while the corresponding echo SPL increased with 2 dB/hd (Fig. 20 C, Fig. 21 C), resulting in an SPL of about 46 dB at the end of buzz 1. Values of absolute emission SPL reduction (resulting from the difference between calculated emission SPL at the beginning of approach and calculated emission SPL at the end of buzz 1) illustrate differences

of SPL depending on different targets: In fact, bats reduced the absolute emission SPL by 17 dB when approaching a single mealworm and a mealworm cluster. However, when bats approached the reflector they reduced absolute SPL only by 11 dB (Table 7).
Using the regression equation I calculated the averaged emission and echo SPL values at the distances of the beginning of approach as well as at the end of buzz 1 for all three target conditions (Fig. 21, Table 7). In the trials with a single mealworm bats had a mean emission SPL at the beginning of approach (1.3 m target distance) of 106.4 dB. During the approach the emission SPL decreased to only 89.4 dB at the position of the end of buzz 1. In trials with mealworm clusters the emission SPL at the beginning of approach (1.6 m target distance) and at the end of buzz 1 were 105.8 dB and 88.5 dB, respectively. Finally, also the emission SPL at the beginning of approach to the reflector (1.7 m target distance) and the emission SPL at the end of buzz 1 were quite similar, with 105.8 dB at the beginning of approach and 94.6 dB at the end of buzz 1 (Fig. 21 C, Table 7). Independent of target type the relations between emission and echo SPL at the end of buzz 1 were quite similar for mealworm, mealworm cluster and reflector approaches with a difference of emission and echo SPL of 53.9 dB in mealworm approaches, 50.1 dB in mealworm cluster approaches and 48.9 dB in reflector approaches. (Fig. 21, Table 7).

The change rates of the emission and echo SPLs during the approaches were quite similar in the single mealworm and the mealworm cluster condition. The different values in the reflector condition (Fig. 21 A-C, Table 7) were not significantly different from the other conditions (ANOVA, emission SPL: $F(2,3) = 2.8841$, $p = 0.2001$; echo SPL: $F(2,3) = 0.6457$, $p = 0.5845$). The change rates also did not differ significantly between the two individuals (emission SPL: t-test = 0.598, $df = 4$, $p = 0.582$; echo SPL: t-test, $t = 0.911$, $df = 4$, $p = 0.4137$).

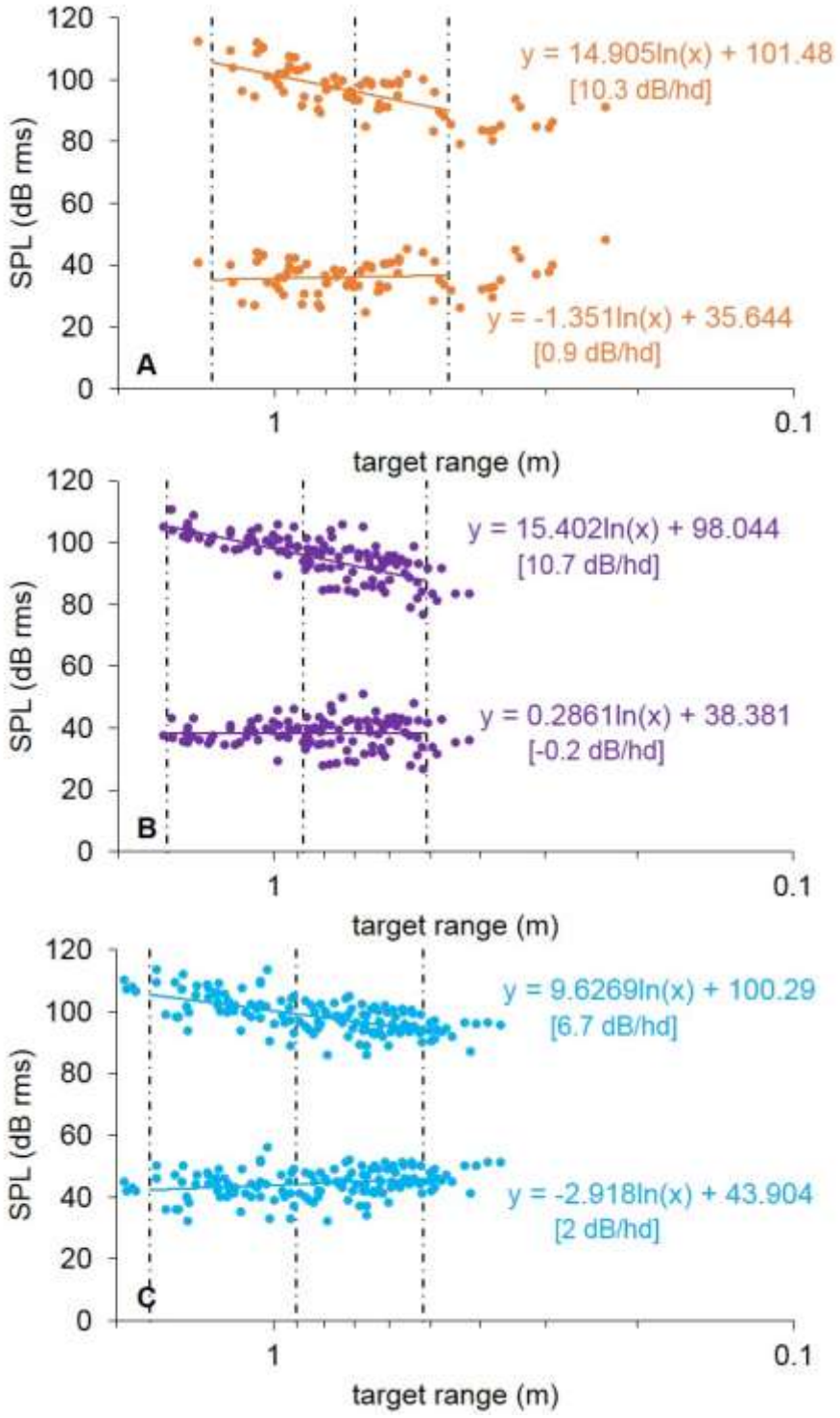



**Fig. 21: Measured echo SPL of *E. fuscus* at the microphone and calculated emission level** during approach for 5 flights per bat in the single mealworm (A), mealworm cluster (B) and reflector condition (C) at a logarithmic range scale. When calculating the emission level I considered the emission level at the microphone to be 10 dB higher than SL. This graph shows the emission and echo SPLs after recalibration according to Weiss (2021) and Koblitz et al. (2010), as explained in paragraph 4.2.3, p. 31. Upper data belong to the emission SPL and lower data to the echo SPL. Resulting SPL changes are written below the formula. Please note that only data from initial approach and buzz 1 are shown. Dashed lines show the mean distances of approach begin, begin of buzz 1 and end of buzz 1.

## 4.4 Discussion

The aim of this study was to compare the motor and echolocation behavior of *Myotis myotis*, a member of the guild of narrow space passive gleaning foragers, and *Eptesicus fuscus*, an edge space aerial-hawking forager, while searching for, approaching, and catching small airborne targets of different target strength. A special aim was to determine how the reduction of the emission SPL during the approach changed the auditory input as described by the relation of the SPL of the emitted signals to the SPL of the returning echoes, which was measured with an on-board microphone near the base of the pinnae.

Narrow space passive gleaning foragers, such as *M. myotis*, have been adapted during evolution to use prey-generated cues for prey detection, classification and localization (Kolb, 1958; Norberg and Rayner, 1987; Arlettaz, 1996; Güttinger, 1997; Arlettaz et al., 2001; Schnitzler and Kalko, 2001; Zahn et al., 2005; Denzinger and Schnitzler, 2004; Denzinger and Schnitzler, 2013). However, *M. myotis* also catch free flying insects and tethered insects and mealworms in a flight room by using the active aerial-hawking hunting mode (Deutschmann, 1991). My experimental procedure was similar to that of Deutschmann (1991) where *M. myotis* had to catch tethered targets. Deutschmann observed in some of the aerial catches that the bats were activated to approach the prey by prey-induced noise. It is very likely that, under certain conditions, *M. myotis* use this foraging mode also in their natural habitat (Arlettaz, 1996; Güttinger, 1997). In the edge space aerial forager *E. fuscus* the aerial-hawking mode is the typical prey catching behavior.

### 4.4.1 Motor behavior of *Myotis myotis* and *Eptesicus fuscus* during search and approach flight and at target capture

All four *Myotis myotis* learned to approach the suspended mealworm and reflector target and to catch it in the aerial-hawking mode. The two *Eptesicus fuscus* were able to catch the three targets (single mealworm, four mealworm cluster and reflector) without a long training besides the training to carry the backpack with recording devices. The capture of the targets during flight can be described as a controlled collision. Unlike the landing situation, where the speed is reduced down to 0 m/sec, the bats reached the aerial target with a certain forward speed. *M myotis* contacted both targets with approximately 1.5 m/s comparable to Natterer's

bats that had an average collision speed of approximately 1.4 m/s when foraging moving prey (Melcón et al., 2007). The collision speed of *E. fuscus* was slightly higher with an average flight speed of 1.9 m/s in the mealworm condition and 2.1 m/s in the reflector condition. In both species the collision speed was roughly at about half of the maximal speed during search flight. Both species formed a pouch with the wing and tail membrane when capturing the different targets. It was also the preferred hunting method when foraging the bigger reflector. Sometimes *M. myotis* also tried to catch the reflector directly with the mouth, a behavior that was also described by Boonman and Jones (2002) when catching small spheres which were mistaken for prey items. Kalko (1995) underlined that she never observed pipistrelles to capture prey with the mouth. She argues that catching the small prey by mouth would require very high localization accuracy. Since the reflector is rather large with a diameter of 1.5 cm it might be possible for the bats to catch it directly with the mouth. Our result shows that the approach flight behavior did not differ with the offered targets and thus were not influenced by any target properties. Other authors (Kick and Simmons, 1984; Faure and Barclay, 1994; Kalko, 1995) also describe pouch hunting on the wing in aerial-hawking vespertilionids. In summary, the two species showed a rather similar motor behavior while catching the offered targets.

### 4.4.2 Echolocation behavior of *M. myotis* and *E. fuscus* during search and approach flight

***Echolocation pattern***

Both species had been trained to search for prey in the confined environment of a flight room and to catch it in the aerial-hawking forging mode while carrying a backpack. The flight room habitat type is acoustically closer to the natural habitats of *M. myotis* who is adapted to forage in narrow space for prey sitting on or flying near background targets as to the natural foraging habitats of *E. fuscus* who uses in the field the aerial-hawking foraging mode to capture prey in open and edge space conditions (Surlykke and Moss, 2000; Hulgard et al., 2016).

In both species I found an echolocation pattern consisting of search phase, initial approach, and terminal approach with buzz 1 and 2 that is typical for aerial-hawking and trawling bats but not for passive gleaning bats (Schnitzler et al., 2003; Schnitzler and Kalko, 2001; Melcón

et al., 2007; Budenz et al., 2018) that normally do not emit buzz 2 (Deutschmann, 1991; Arlettaz et al., 2001). Flick (2008) reports in her diploma thesis that in passive acoustic prey catching *M. myotis* buzz 2 could sometimes be observed when landing on a food site. Also Arlettaz et al. (2001) and Russo et al. (2007) report buzzing *M. myotis* when gleaning from the substrate. This is explained to be the short and loud so-called landing buzz which is widespread among gleaners, which conveys information for a successful landing and does not assist in localizing prey. When foraging in the flight room *M. myotis* and *E. fuscus* showed the typical sound emission pattern of aerial-hawking bats.

***Search phase***

The pattern of search signals in the two species was rather similar. During search they emitted per wing beat either one or two wideband FM signals with an end frequency near 28 kHz and an average duration between 1.4 – 2.2 ms in *M. myotis* and a slightly longer duration of 2.3 – 2.6 ms in *E. fuscus*. The PI in situations, where most probably two signals were emitted per wing beat, was around 52 ms in *M. myotis* and around 52 – 75 ms in *E. fuscus*. The pattern of *M. myotis* was similar to that of other studies where *M. myotis* was studied in a flight room (Deutschmann, 1991; Flick, 2008; Budenz, 2018). In *E. fuscus* the search pattern in the flight room differed strongly from that of bats while searching for prey in open space, where calls are much longer, louder and more narrowband and emitted either every or every second wing beat (PD 14 – 20 ms, PI 134 or 270 ms; Surlykke and Moss, 2000), but it was comparable to other recordings from *E. fuscus* in comparable flight room conditions (Surlykke and Moss, 2000; Moss and Surlykke, 2001; Sändig et al., 2014; Saillant et al., 2007).

***Approach phase***

Both species showed the typical approach behavior of edge space bats that catch airborne prey. In both species a reduction of pulse duration and interval indicated the beginning of the approach during which the duration of PD and PI was reduced. The approach ended with a long terminal group or buzz which could be subdivided in buzz 1 and buzz 2 according to a buzz 2 part where the PI stayed constant at about 6 ms, where the end frequency was reduced, and where the shortest signals were emitted. The beginning of the approach depended on

target strength. *M. myotis* reacted to the loud target, the reflector, in a larger detection distance (1.2 ± 0.1 m) than they did for the weak target, the mealworm (0.96 ± 0.08 m). In *E. fuscus* the reaction distance also increased with rising target strength from 1.32 m at one mealworm, to 1.61 m at 4 mealworms, and to 1.73 m at the reflector target. Thus, our results concur with the findings of Budenz et al. (2018) where gleaning *M. myotis* started the approach to a loud reflector dish in a greater distance than to a lower foam dish.

During the approach *M. myotis* and *E. fuscus* arranged their echolocation calls in groups with an increasing number of calls within a group the smaller the distance to the target. The grouping was independent of target type. I assume that an increase of the numbers of signals in a group may lead to a more precise localization of targets by processing each group as a package (Moss and Surlykke, 2001; Moss et al., 2006; Sändig et al., 2014).

The two-way travel time indicates how long it takes for a sound sent out and the corresponding echo thrown back from the target to get from the bat via the target back to the bat. Bats approaching a target have the problem that the masking of the target echo by the emitted call or by subsequent clutter echoes can limit the bats' ability to process the information contained in pulse-echo pairs: For close targets the emitted signal will overlap with the target echo, thus creating a forward-masking effect and when the target is close to the background the echoes from background will overlap with the target echo itself, creating a backward-masking effect. If the PI stays below the two-way travel time, the emitted sounds will not overlap with the returning echo and a masking effect will be avoided. Although *M. myotis* is not an aerial forager under normal circumstances, in my study it almost always avoided an overlap of outgoing pulses and returning echoes, as is often observed in bats foraging insects in the active mode (Kalko and Schnitzler, 1989, 1993; Denzinger and Schnitzler, 2013). Overlapping was also avoided by *E. fuscus*.

### *Buzz 1 and 2*

*M. myotis* and other passive gleaning bats approaching a site with food do not emit a distinct terminal group or buzz (Deutschmann, 1991; Schnitzler and Kalko, 2001; Schnitzler et al., 2003; Russo et al., 2007; Arlettaz et al., 2001; Melcón et al., 2007; Flick, 2008; Budenz et al.,

2018). I found that *M. myotis* catching suspended targets in the aerial-hawking mode emitted an approach sequence with a prominent terminal part consisting of buzz 1 and buzz 2, just like the aerial-hawking *E. fuscus* did. Buzz 1 is typically characterized as the first part of the long terminal group where PI and PD are still continuously reduced. In contrast, buzz 2 typically shows a constant PI at values of approximately 6 ms and minimal PD (Griffin et al., 1960; Siemers and Schnitzler, 2000; Melcón et al., 2007). In the meantime, the meaning of buzz 1 during insect catch is well understood: for example, an increased repetition rate serves to optimize information flow and thus increases localization accuracy. By reducing the pulse duration, on the other hand, the overlap-free window is maintained because the overlap of outgoing echolocation call and the returning echo is avoided (Melcón et al., 2009; Denzinger and Schnitzler, 2013). Buzz 2 usually starts so shortly before the insect catch that the bat no longer has time to process the information it receives and to react by changing its position or direction (Kalko, 1991; Melcón et al., 2009). According to Kalko (1991), the reaction time for vespertilionids is between 80 and 100 ms. Hence, buzz 2 does not seem to have any relevance for a successful prey capture and I did not further investigate buzz 2 in my work. Melcón et al. (2007) hypothesize that buzz 2 may help to understand what went wrong in an unsuccessful insect catch.

***Assessment of the approach behavior of the two species when catching airborne prey***

So far I defined the begin of the approach phase as the part of a capture sequence where the bat reacted to the detected target by reducing sound duration and pulse interval and by lowering the emission SPL. This reduction range of the approach started in *M. myotis* immediately after the detection of the target. In *E. fuscus*, however, the reaction distance for the reduction of sound duration, pulse interval, and emission SPL was distinctly shorter than the detection distance. For instance, the calculated detection distance for the single mealworm was 2.5 m (range calculator of Stilz and Schnitzler (2012): http://u-017-s090.v263.uni-tuebingen.de/~peter/open/calculator/range.php, see p. 75 et seq.) whereas the reduction range started at 1.32 m. According to our working hypothesis that the reduction of the emission SL has the adaptive function to adjust the ratio between emission and echo SPL level in the optimal range of the hearing system for precise range measurements I will discuss my results under this aspect. Both species showed in the reduction range of the approach a rather similar sound pattern which is also found in other aerial-hawking bats and which allowed to

discriminate an initial part from a terminal part with buzz 1 and 2. However, the SPL reduction started from different levels and was in *E. fuscus* about 16 dB above the level of *M. myotis*, and the lowering rate of the emission SPL was clearly different. These distinct differences may reflect that *E. fuscus* and *M. myotis* occupy different ecological niches and need therefore a different adaptive echolocation behavior for the adjustment of the SPL ratio of emitted sound and echo in the optimal range. This will be the topic of the following discussion.

***Reduction of the emission SPL as a measure for the beginning of approach***

Both species reduced the emission SL during the approach but started from different SPLs of their search signals. In *M. myotis* the start of the reduction defined by a change of 2 standard deviations from the values of the search signals did not differ significantly from the beginning of the approach defined by a reduction of pulse duration and pulse interval for none of the conditions. However, bats reacted with an SPL reduction at a greater distance to the loud reflector target (1.25 ± 0.2 m) as to the weak mealworm target (1.03 ± 0.17 m). This shows that bats not only react with a reduction of sound duration and pulse interval when starting the approach but also with a reduction of SPL. *E. fuscus* also reduced the emission SPL during the approach. I only got echoes when the bat was with its sonar beam on the target. The distance, when I recorded the first echoes and the distance, when the approach started according to the reduction of pulse interval and pulse duration again did not differ significantly in any of the conditions, although the distance between conditions again depended on the target strength (single mealworm 1.3 m, mealworm cluster 1.6 m, reflector 1.7 m. From this I conclude that the first recorded echoes coincide with the beginning of the approach.

For *Pipistrellus kuhlii* it has been shown that the approach starts when the bat detects a target (Schnitzler et al., 1987). With the parameters SL, TS, C2, and signal frequency I determined for both species the detection distance for the different targets for an assumed detection threshold of 20 dB by using the echolocation range calculator of Stilz and Schnitzler (2012) (http://u-017-s090.v263.uni-tuebingen.de/~peter/open/calculator/range.php). In *M. myotis* the calculated detection distances of 1.1 m at the mealworm target and of 1.5 m at the reflector

were rather close to the measured reaction distances of 1.03 resp. 1.25 m. That means that *M. myotis* started the approach immediately after the detection of the targets. In *E. fuscus* the calculated detection distances of 2.5 m for the single mealworm, 3.4 m for the cluster and 5.1 m for the reflector are distinctly above the measured reaction distances of 1.32, 1.61 and 1.73 m which suggests that the bats did not react with approach behavior immediately after detection. That leaves me with the question what triggered the beginning of the approach behavior in this species and also in other bats that use search signals with a similarly high SL.

***Source level and ratio of emission and echo SPL at the pinna***

A distinct difference between flight room and natural foraging situations of bats is the SL of the emitted signals. For instance, in *E. fuscus* that forage in open space, SLs around 111 dB rms re 1 m with maximum values of 118 dB (Hulgard et al., 2016) have been measured which corresponds well with the data from other open space species (summarized in Stilz and Schnitzler, 2012). Unfortunately, the SL of *E. fuscus* while foraging in the flight room could not be measured directly due to methodological problems. However, it is known from *E. fuscus* flying in comparable flight room situations that they emitted signals with a SL near 96 dB rms re 1 m (Koblitz et al., 2010; Weiss, 2021). Therefore, I concluded that in my experiments the SPL during search flight was also 96 dB rms re 1 m and I used this value to recalibrate my SPL measurements. The assumption that *E. fuscus* flying in confined spaces use a SL around 96 dB is additionally confirmed by the statement of Hulgard et al. (2016) that SL estimates based on field recording of bats foraging in open space are 15 – 20 dB more intense than that based on laboratory data.

Narrow space bats emit lower SL than edge and open space bats (Brinklov et al., 2009; Jakobsen et al., 2013). In the rather confined environment of the flight room comparable to the acoustic situation near background targets in narrow spaces I measured a SL during search flight of 80 dB rms re 1 m in *Myotis myotis*. This value is close to the SL of about 76 dB measured in *M. myotis* in a flight room when searching for mealworms in a dish on the ground (Budenz et al., 2018).

The SL refers to the SPL measured at 1 m in front of the bat's mouth. I assume according to the measurements of Henson (1965) that the emission SPL at the pinna of a bat as measured

with the Telemike is about 10 dB higher than the SL measured at 1 m in front of the bat. In the further discussion all given SPL values refer to the SPL at the pinna. The mean values of the emission SPL at the pinna is thus 106 dB in *E. fuscus* and about 90 dB in *M. myotis* in search flight.

My measurements allow to compare the behavior of two species from two different guilds, a passive gleaning forager and an edge space aerial forager, which have to solve a similar echolocation task and which emit a similar pattern of search signals but differ in the SL of their search signals by about 16 dB. I explain this difference as an adaptation to the preferred foraging habitat according to the rule: The more bats hunt in the open, the louder and longer and more narrowband are their signals, the lower is the emission frequency, and the larger are their detection distances (Schnitzler et al., 2003; Stilz and Schnitzler, 2012). The comparison of the approach behavior of the two species showed that they differed in the absolute reduction of the emission SPL and that they adjusted the emission and the echo SPL in a different way (Fig. 15 and Table 3 for *M. myotis* and in Fig. 21 and Table 7 for *E. fuscus*).

Both species reduced the emission SPL during approach but with different reduction rates at the different targets. In *M. myotis* the emission SPL when approaching the smaller target was reduced with a minimal higher rate in comparison to the reduction rate when approaching the larger target. By this difference in steepness *M. myotis* compensated for the different detection distances so that the emission SPL at the end of buzz 1 was in the same range independent of target size. In *E. fuscus* the reduction rate of the emission SPL was much higher than in *M. myotis*. The higher SL in *E. fuscus* resulted in an earlier reaction as in *M. myotis* and also in a different change rate of the echo SPL during the approach. Additionally, reduction rate was, as a rule, higher in the smaller targets. In contrast to *M. myotis* the echo SPL stayed about the same at all three target types. All the changes together led at the end of buzz 1 in *M. myotis* to a ratio of emission to echo SPL at the pinna of 75.2 : 37.4 dB (mealworm target) and 70.8 : 35.5 dB (reflector). In *E. fuscus* pulse-echo pair ratios of 89.4 : 35.5 dB (one mealworm), 88.5 : 38.4 dB (4 mealworms), and 94.6 : 45.7 dB (reflector) have been measured.

Unfortunately, the only existing data on the emission and echo level at the approach to airborne prey in aerial-hawking species are not directly comparable to my data as the authors measured the energy and not the SPL of the emitted signals and the returning echoes.

Nevertheless, on trend they came to a result which compares well with our data. *Myotis myotis* lowered during the approach the energy level of the emitted signals so that the echo level was slightly increased (Stidsholt et al., 2021, Fig. 3) and in *Eptesicus fuscus* the higher emission level and distinct reduction of the emission level kept the echo level about constant throughout the whole approach (Stidsholt et al., 2018, Fig. 6). In another approach Saillant et al. (2007) studied *E. fuscus* when catching a tethered small microphone. In one bat the SPL of the recorded signals stayed constant during the approach which indicates that the emission SPL was reduced with 6 dB/hd. In another bat the SPL of the recorded signals was reduced thus indicating a reduction rate above 6 dB/hd which would correspond to my data.

### 4.4.3 The optimal SPL ratio of pulse-echo pairs for precise range measurements

From psychophysical and neurophysiological studies it is known that the relation of emission to echo SPL has a strong impact on the ranging abilities of bats. The accuracy with which the bats can determine the time delay between emitted signal and returning echo and with it the target range depends on this relation. In a range discrimination task with phantom targets (S+ was at a distance of 52.7 cm, and the echo SPL varied between -8 and -48 dB relative to emission SPL of about 90 dB at 13 cm in front of the bats' mouth (Denzinger and Schnitzler, 1994)) the ranging performance of *E. fuscus* was best if the echo SPL at the ears of the bats was -28 dB below the emission level which was at about 90 dB at a distance of 13 cm front of the bat. The bats' performance worsened at higher and lower echo SPLs. The perceived emission SPL at the cochlear input is influenced by the attenuation effect of the contractions of the middle ear muscles during vocalization, and the directionality of sound emission which has the effect that the SPL at the ear is lower than the SPL in front of the bat (Henson, 1965). Budenz et al. (2018) estimated that the perceived emission SPL in the ranging experiments was approximately 28 dB lower accounting an attenuation effect due to the MEM effect by about 18 dB and the directionality of sound emission by about 10 dB (data from Henson, 1965). That means that in the ranging experiments *E. fuscus* perceived the emitted signals with an SPL of 62 dB. The perceived echo level which produced the best performance is 28 dB lower than the emission level of 90 dB plus the attenuation produced by the middle ear reflex at the S+ target distance of 52.7 cm. Patheiger (1998) who studied in *Eptesicus fuscus* how the vocal middle ear reflex affects the detection threshold for a wire target offered at

different distances determined that echoes returning from a distance of 52.7 cm were attenuated by about 6 dB. This means that the ranging performance measured by Denzinger and Schnitzler (1994) was best at a perceived emission SPL of 62 dB and a perceived echo SPL of about 56 dB (90 – 28 – 6 dB). According to this estimation *E. fuscus* would have reached the best ranging performance if the perceived echo level was just below the perceived emission level namely at a SPL relation of the perceived pulse-echo pairs of about 62 : 56 dB.

The importance of SPL relations of pulse-echo pairs for the ranging performance is also documented by several neurophysiological studies on ranging mechanisms of bats using FM signals (summarized in Budenz et al., 2018). Here, a loud FM signal simulating a bat's emitted pulse followed by a weak FM signal simulating a returning echo was used as a range stimulus in *Myotis lucifugus* (Sullivan, 1982; Berkowitz and Suga, 1989) and *Pteronotus parnellii* (Berkowitz and Suga, 1989). The authors tested how the time delay between pulse and echo encodes target range. In *M. lucifugus*, cortical neurons reacted with a longer response latency to strong signals than to weak signals. This is called paradoxical latency shift (Sullivan, 1982). The difference between the two corresponds to the best delay of the neuron. However, these shifts occurred only at specific stimulus amplitudes: The weak stimuli had an upper limit of 29 – 53 dB SPL and the strong stimuli a lower limit of 62 – 84 dB SPL. This suggests that this ranging mechanism only works if the emission SPL is above 62 dB and the echo SPL below 53 dB. Hechavarria and colleagues (2013) also showed that the SPL of pulse-echo pairs is important for the ranging performance of bats when they recorded from echo-delay coding neurons in the cortex of three bat species (*Pteronotus parnellii*, *P. quadridens* and *Carollia perspicillata*).

In summary, behavioral and neurophysiological studies suggest that the ranging performance of bats depends on the absolute SPL and on the relative SPL ratio of pulse-echo pairs with a best working range at perceived emission SPLs of about 60 – 80 dB and perceived echo SPLs of about 40 – 60 dB (Budenz et al., 2018; Denzinger and Schnitzler, 1994, 1998; Berkowitz and Suga, 1989; Hechavarria et al., 2013).

I assume that this adaptation of bats to an optimal working range at SPLs between 40 – 80 dB reflects a general constraint set by the mammalian hearing system which – independently of species – performs best if the SPLs of signals are within this dynamic range. For instance, the

dynamic range of speech in humans is around 40 dB at absolute SPLs between 40 – 80 dB (National Research Council, 2005).

The localization accuracy of the prey directly after detection is not optimal according to the unfavorable ratio between perceived emitted and echo SPL. At an assumed detection threshold of 20 dB the ratio of the pulse-echo pair SPL at the pinna would be 90 : 20 dB in *M. myotis* and 106 : 20 dB in *E. fuscus* in the flight room experiments. For *E. fuscus* foraging in open space an even more unfavorable SPL ratio at the pinna of 121 : 20 dB has to be assumed. All values are clearly outside of the optimal processing range of a mammalian hearing system (Budenz et al., 2018). Nevertheless, they seem to be sufficient for a rough localization of the prey for the bats are able to fixate it after detection and to make a directed approach to it. However, for the control of the flight speed reduction for a controlled collision with the prey and for the planning of adequate capture movements bats must improve their localization accuracy with decreasing distance to the prey. I argue that this is the reason why bats react after the detection of a target of interest with a specific approach behavior in which emission and echo level are adjusted to the optimal ratio for precise range measurements.

### 4.4.4 SPL ratio of perceived pulse-echo pairs at the cochlear input

The boundary conditions for the adjustment of the SPL ratio of the perceived pulse-echo pairs depend on several factors. The echo level increases during the approach to a target. The increase rate is determined by distance dependent acoustic effects namely atmospheric attenuation and geometric spreading (Stilz and Schnitzler, 2012). Geometric spreading depends on the reflection properties of the target of interest. In an insect target (comparable in a first approximation to a point target) the echo level increases with values near 12 dB/hd and in an extended landing site (comparable to a mirror target) with 6 dB/hd. The echo increase rate during an approach is additionally influenced by the corresponding reduction rate of the emission level. For instance, in *E. fuscus* the steep echo increase is compensated by a corresponding steep reduction of the emission level so that the echo SPL is kept near 40 dB during the whole approach. In *M. myotis* where the low SL produces weak echoes the echo level still rises throughout the whole approach from about 20 to about 40 dB which results from the small reduction rate of the emission SPL. In summary, the detection distance, the

echo increase rate and the reduction rate of the emission SPL during approach are the factors which determine the echo SPL at the pinna at the end of the approach.

However, I need to know the perceived emission level at the input of the cochlea if I want to judge whether the approach behavior has the function to adjust the ratio between emission and echo level to the optimal range for precise distance measurements.

The perceived emission SPL at the input of the cochlea depends on the SL, the crosstalk attenuation between the bats' mouth and ears, and the attenuation effect of a potential MEM reflex. The SL of bat calls is measured at 1 m distance in front of the bat in the middle of the directional emission beam. The 10 cm SPL is 20 dB above the SL at 1 m. The crosstalk between emitted signal and signal SPL at the base of the pinna has been estimated from measurements of microphonics in *Tadarida brasiliensis* to be about 10 dB below the SPL measured at 10 cm in front of the bat (Henson, 1965; Budenz et al., 2018). Measurements in *Nyctalus noctula* revealed a value of 13 dB (Stidsholt et al., 2018). The pinna SPL is thus 10 – 13 dB higher as the SL. Here I used this argument to determine in *M. myotis* the SL by reducing the measured Telemike SPL by 10 dB according to Henson (1965). In *E. fuscus* I determined the pinna level by adding 10 dB to the assumed SL. However, a look on the faces of bats shows that the morphological relations between the transmitter antenna (open mouth) and the receiver antennae (forward directed ears) are highly different between species which suggests that the size of the crosstalk loss from mouth to ears will also differ between species. I hypothesize that it will be larger in bats which use high SL for long range echolocation to prevent an overload of the hearing system by too loud perceived emissions SPLs. The value of 10 dB which is derived from the *Tadarida* data of Henson (1965) and the 13 dB measured in the European noctule may therefore be a rather rough approximation but is most likely species specific.

The middle ear reflex is another possibility to prevent the overload of the hearing system by attenuating incoming loud signals. It has been already mentioned that the MEM reflex of the open space forager *Tadarida brasiliensis* had an attenuation effect of about 20 dB at the beginning of signal emission (Henson, 1965). It has also been shown by electrophysiological studies in humans (Borg and Zakrisson, 1975) that the size of the MEM attenuation depends on the absolute SPL. For instance, a reduction of emission SPL from 100 to 70 dB strongly

reduced the activity of the middle ear muscles thus indicating a reduced attenuation of the vocal MEM reflex. Unfortunately, in bats data on the relation between size of the emission SPL and attenuation effect of the MEM reflex are lacking. However, it is very likely that bats which use loud search signals have a stronger MEM reflex as bats with lower emission SPLs.

At close target distances the MEM reflex not only attenuates the emission SPL but also the SPL of echoes that return shortly after signal emission. For instance, I know from measurements of the detection threshold of *E. fuscus* at different target distances (Patheiger, 1998) that the attenuation effect was still about 6 dB at a target distance of 0.5 m. This negative effect could be reduced or even abolished if the lowering of the emission SPL during the approach lead to a reduction or even omission of the MEM reflex. This is not unlikely as the size of the MEM reflex depends on the absolute SPL of the vocalization which causes it (Borg and Zakrisson, 1975).

For a rough estimation of the perceived emission SPL of *E. fuscus* I make the following assumptions. The emission SPL of *E. fuscus* is loud enough to cause a strong MEM reflex which attenuates the pinna SPL of about 106 dB at approach begin to an about 20 dB lower perceived emission SPL somewhere around 86 dB (Henson, 1965). The emission SPL reduction during the approach most likely results in an additional reduction of the MEM reflex (Borg and Zakrisson, 1975). The pinna SPLs between 95 – 88 dB at the end of buzz 1 are therefore less attenuated (maybe only 10 dB) so that the perceived emission SPL would be around 85 – 78 dB. The echo levels during the whole approach were not attenuated so that the perceived echo levels stayed about constant within 35 – 46 dB. According to this rather speculative explanation attempt I assume that the approach behavior of *E. fuscus* has the function to adjust the relation of the perceived emission to echo SPL from the beginning of the approach until the end of buzz 1 at an about constant ratio of 85 – 78 dB to 35 – 46 dB which is still in the range where precise range measurements are possible.

*M. myotis* with their 16 dB lower SL most likely have a weaker or no MEM reflex so that the attenuation effect should be smaller. For a rough estimation I assume that the MEM reflex is around 10 dB at the beginning of the approach and 0 dB at the end of buzz 1. With this assumption the perceived emission SPL would be about constant throughout the whole approach with values between 75 – 70 dB. *M. myotis* started with the approach behavior

immediately after detection so that the echo levels at the beginning of the approach were with approximately 20 dB still rather low. During the approach echo levels increased with 6.1 and 7.9 dB/hd rather steeply so that they reached values near 35 – 37 dB at the end of buzz 1. With these assumptions the approach behavior of *M. myotis* would have the function to adjust the perceived pulse-echo SPL ratio of about 75 – 70 to 20 dB at approach begin to about 75 – 70 to 35 – 37 dB at the end of buzz 1 which brings the ratio into the range where precise range measurements are possible.

I am aware that our assumptions concerning the factors which determine the perceived emission and echo level are rather speculative. For a conclusive argumentation on how species adapt their approach behavior I would need measurements of the size of the crosstalk between the bat's mouth and pinnae and also of the attenuation effect of the level dependent MEM reflex.

With the exception of the *Tadarida* measurements with 10 dB crosstalk loss between pinna input and 10 cm SL (Henson, 1965) and data from *Nyctalus noctula* with a loss of 13 dB between the microphone on the back and the 10 cm SL (Stidsholt et al., 2018) I have no information on the size of the species-specific crosstalk between the bats mouth and pinnae. I also know nothing about the emission level dependent size of the MEM reflex in different species. However, from comparative echolocation studies I know that the overload risk of the hearing system strongly differs according to the preferred search ranges of species. Species which are adapted for long range echolocation have much higher search levels (reviewed in Stilz and Schnitzler, 2012) than species adapted for medium and short ranges. Even within species the SL varies according to the search conditions, for instance in *E. fuscus* by up to 15 – 20 dB (Hulgard et al., 2016).

The fact that the ranging performance of all bats is best if the perceived emission SPLs are within about 60 – 80 dB and the perceived echo SPLs within about 40 – 60 dB (Budenz et al., 2018; Denzinger and Schnitzler, 1994, 1998; Berkowitz and Suga, 1989; Hechavarria et al., 2013) requests in bats with different emission SLs different SPL adjustment strategies during target approach. Bats with a high SL (for instance in bats foraging with 111 dB in open space) need a strong SPL reduction during the approach to adjust the perceived emission SPL within the optimal range for distance measurements.

For instance, a distinct crosstalk reduction (-15 dB) in combination with a strong MEM reflex (-25 dB) would lead to a perceived emission SPL of 91 dB at the beginning of the approach, and the optimal range would be reached with an additional SL reduction during the approach. In bats with a medium SL (e.g. in *E. fuscus* foraging in a flight room with 96 dB) the reduction can be smaller. For instance, a crosstalk reduction (-10 dB) in combination with the MEM reflex (-20 dB) would lead to a perceived emission SPL 86 dB at the beginning of the approach, and the optimal range would be reached with an additional medium sized reduction of the emission SL during the approach. In bats with a low SL (e.g. *M. myotis* foraging in a flight room) the SPL reduction during approach can be smaller. For instance, in a bat with a low SL (80 dB) a crosstalk reduction (still -10 dB) in combination with a moderate MEM reflex (-10 dB) would lead to a perceived emission SPL 80 dB at the beginning of the approach, so that an additional small reduction of the emission SL during the approach would adjust it in the optimal range.

In summary, the comparison of the two ecologically different species indicates that the approach behavior of species with different search distances must be different. Long range bats emit very loud signals and need therefore a large SPL attenuation to adjust the perceived emission SPL in the optimal working range for distance measurements. Short range bats emit weak signals so that the attenuation can be small. However, they have the problem that weak signals produce weak echoes so that their emission SPL attenuation should be so small that the echo increase rate is still large enough so that the echo SPL can reach the optimal processing range. The approach adjustment of medium range bats has to be somewhere in between.

Unfortunately, I do not yet have the data which could support this hypothesis. I should know more about the SL, the crosstalk attenuation, the size of the middle ear reflex, its SPL dependent variation, its reduction during the approach, and the size of the echo SPL and its increase rate in ecologically different species while foraging for prey. All this information would be delivered by telemetric recordings of the microphonics of behaving bats.

### 4.4.5 Mechanisms for the emission SPL adjustment during target approach

Most studies published so far imply that the reduction of emission SPL regulates the echo SPL in a closed loop or feedback control system. The proposed mechanisms are “automatic gain control” (Kick and Simmons, 1984), “intensity compensation” (Kobler et al., 1985) and “dual component system for stabilization of the perceived echo amplitude” (Hartley et al., 1992 a, b). However, such a closed loop control system requests that the perceived echo SPL is kept constant at a specific reference level (Budenz et al., 2018). Boonman and Jones (2002) who studied the approach behavior of *M. daubentonii* that were approaching tethered spherical targets differing in TS and Budenz et al. (2018) who studied the approach behavior of *M. myotis* when foraging in the passive mode for rustling mealworms offered in feeding dishes with different target strength found that the lowering of the emission SPL does not lead to constant echo levels which rejects the proposed theories for intensity compensation.

My studies with *E. fuscus* and *M. myotis* deliver a mixed picture. In *E. fuscus* the echoes of all three targets were kept about constant around 40 dB throughout the whole approach until the end of buzz 1. The differences in TS were compensated by different reaction distances and different reduction rates of the emitted SPL. At all three targets the echo levels were at the beginning of the approach phase between 35 and 42 dB which suggests that this is the threshold which triggered the release of the approach phase. In *M. myotis* approach phase of the two target types started at echo levels around 20 dB. However, during approach a rather low reduction rate of the emitted signal resulted in an echo level increase to 35 – 37 dB at the end of buzz 1.

The sensory feedback mechanisms which are responsible for the two different ways to adjust during approach the emission and echo SPL as described here for *E. fuscus* and *M. myotis* are not known. For sure it is not a simple feedback system which keeps the echo SPL constant at a reference value as it is implicitly assumed by the current views concerning the echo level adjustment such as automatic gain control, intensity compensation, and dual component system. The antipode to the closed loop control system would be an open loop control system, where the control action is completely independent of the controlled process variable. This would imply an uncontrolled and stereotyped reduction of the approach speed and of the emission SPL. I agree with Budenz et al. (2018) that this mechanism is rather unlikely. Geberl

and colleagues (2015) made an interesting experiment with *M. daubentonii* by removing the prey just before the expected catch. They found that the bats respond to the sudden removal of the prey with a delayed omission of otherwise important parts of their sensory-motor behavior (emission of buzz 2 and typical capture behavior with hind legs/wings). In this way they showed that the approach is under feedback control. Also Amichai and Yovel (2017) showed in an interesting experiment that landing *Pipistrellus kuhlii* adapt their approach behavior to the distance of the target. This also suggests a distance-dependent sensorimotor feedback control. However, the feedback control system for adjusting approach behavior is rather slow (Budenz et al., 2018). Geberl et al. (2015) estimated reaction times of about 80 ms and Denzinger and Schnitzler (personal communication) measured 40 – 60 ms in foraging bats. These times still exclude a fast feedback reaction. From the data of Koblitz et al. (2011) I conclude that it takes as long as about one sound group or one wing beat, until a bat adapts its echolocation behavior according to the new information from the previous group of sounds. I agree with Budenz et al. (2018) that there are good arguments that bats use acoustic flow field parameters not only for the guiding of the controlled collision with the prey but also for the adjustment of the perceived emission and echo SPL at the optimal processing range of the hearing system. The use of acoustic flow field information in bats has first been described by Lee and colleagues (1992, 1995) who showed that bats use acoustic flow field parameters when slowing down to catch a mealworm (controlled collision) and when braking to land on a wall in order to control their approach speed. Further evidence comes from studies that show that bats' flight is in fact affected by echo-acoustic flow (Kugler et al., 2016; Warnecke et al., 2016) and electrophysiological studies revealed neurons in the bats' auditory cortex that selectively respond to echo-acoustic flow fields (Bartenstein et al., 2014; Beetz et al., 2016; Greiter and Firzlaff, 2017). It may thus be that bats use acoustic flow field parameters not only for controlling their flight speed and distance to targets but also for feedback controlling of the emission SPL during target approach.

### *Emission and echo level adjustment during approach in landing bats*

During an approach the change rate for the amplitude of outgoing pulses and returning echoes is measured in dB/hd which is a distance-dependent measure and not a temporal one. It results, however, from time-dependent behaviors of the bats: the deceleration of the bat when approaching a target or a landing site as well as from the change of emission SPL during the

approach towards the same (Budenz et al., 2018). For many bat species and in approaches to various targets an emission SPL reduction rate of about 6 dB/hd has been reported (Griffin, 1958; Jen and Kamada, 1982; Kobler et al., 1985; Hartley et al., 1989, 1992 a, b; Tian and Schnitzler, 1997; Boonman and Jones, 2002; Hiryu et al., 2007, 2008; Melcón et al., 2007, 2009; Saillant et al., 2007; Brinklov et al., 2009, 2010; Koblitz et al., 2011; Norum et al., 2012). The rather uniform result that the landing bats reduced the emission SL by about 6 dB/hd may result from the fact that the tested species used rather similar emission SPLs before they started the approach to the landing site. Further studies have to show whether bats which start from a higher or lower emission SL differ in their approach behavior. In a recent study, Stidsholt and colleagues observed *Nyctalus noctula* when landing on a nylon target sphere (Stidsholt et al., 2018). In this study, the authors only measured the energy of echolocation calls and echoes so the reduction rate in dB/hd is not available.

A distance dependent reduction rate in dB/hd might be advantageous for the bats as it delivers information on the nature of targets. The reflection properties of large targets are close to those of acoustic mirrors and cause an echo SPL increase rate of 6 dB/hd. Thus an emission SPL reduction of 6 dB/hd at mirror like targets, would completely compensate for the echo increase rate of 6 dB/hd and the echo SPL would be kept constant but 6 dB lower at the bat's ear. In contrast, point targets and small targets evoke an echo SPL increase of 12 dB/hd. Here, an emission SPL reduction rate of the same 6 dB/hd would compensate only partly for the echo increase rate and thus result in an echo SPL increase rate of 6 dB/hd at the bat's ear. An increase rate of 3 dB/hd indicates a wire or another cylindrical target.

## 4.5 Conclusion

The comparison of the echolocation behavior of *M. myotis* and *E. fuscus* confirmed the working hypothesis that bats using the aerial-hawking mode to capture tethered prey adjust during target approach - independent of the SL of their search signals and independent of the target strength of their prey - the ratio of the perceived SPL of emitted signals and returning echoes to the optimal range of the hearing system for precise distance measurements. At the end of buzz 1 at an average target distance between 17 – 19 cm *M. myotis* reached at both target types ratios within 75 – 70 dB emission SPL to 37 – 35 dB echo SPL whereas *E. fuscus* where buzz 1 ended rather early at distances around 50 cm reached at the three target types ratios within 95 – 88 dB emission SPL and 46 – 35 dB echo SPL. At a distance of 30 cm for the end of buzz 1 which was reached at some approaches *E. fuscus* reached calculated ratios within 89 – 80 dB for the emission SPL and 47 – 37 dB for the echo SPL (compare Fig. 21).

The adjustment phase of the approach which is characterized by a reduction of pulse duration, pulse interval and emission SPL began in *M. myotis* immediately after target detection and started at a target distance of either 103 cm (at the mealworm target) or 125 cm (at the reflector target) and in each condition at an echo SPL of approximately 20 dB (see Fig. 15). In *E. fuscus*, which emit search signals with a SL 16 dB higher than that of *M. myotis*, the calculated detection distance was distinctly further out than the beginning of the adjustment phase of the approach. Here the start of the adjustment phase also differed according to the different target strength of the prey items between 132 – 173 cm and was triggered when the echo level at the microphone of the three target types surpassed 35.3 dB, 38.5 dB and 42.3 dB (see Fig. 21). Different SLs of the search signals and different echo levels that trigger the adjustment phase resulted in different reduction rates of the SL to reach the optimal ratio of emission and echo level for distance measurements. In *M. myotis* with its low search SL the reduction rate for the emitted signals was rather low which resulted in a distinct echo increase to about 40 dB. In the louder *E. fuscus* the reduction rate was rather high which kept the echo level constant near about 40 dB. These results support the hypothesis that the adjustment behavior of bats approaching prey is adapted in each species to the echolocation conditions of their ecological niche. Bats with high search SL have high reduction rates and keep the echo SPL rather constant near 40 dB whereas bats with low search SL have lower reduction rates of the emitted signals so that the echo SPL is raised to about 40 dB. The measured data do not

allow making conclusions about the perceived SPL ratios at the cochlea since it is not known how the middle ear reflex effects the auditory input during the approach.

## 4.6 References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