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# An integrative approach to detect subtle trophic niche differentiation in the sympatric trawling bat species *Myotis dasycneme* and *Myotis daubentonii*

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## Abstract

Bats are well known for species richness and ecological diversity thus they provide a good opportunity to study relationships and interaction between species. To assess interactions we consider distinct traits which are likely to be triggered by niche shape and evolutionary processes. We present data on the trophic niche differentiation between two sympatric European trawling bat species, *Myotis dasycneme* and *M. daubentonii*, incorporating a wide spectrum of methodological approaches. We measure morphological traits involved in foraging and prey handling performance including bite force, weight lifting capacity and wing morphology. We then measure resulting prey consumption using both morphological and molecular diet analysis.

These species closely resemble each other in morphological traits however, subtle but significant differences were apparent in bite force and lift capacity which are related to differences in basic body and head size. Both morphological and molecular diet analyses show strong niche overlap. We detected subtle differences in less frequent prey items, as well as differences in the exploitation of terrestrial and aquatic-based prey groups. *M. dasycneme* feeds more on aquatic prey, like Chironomidae and their pupal stages, or the aquatic moth *Acentria ephemerella. M. daubentonii* feeds more on terrestrial prey, like Brachycera, or Coleoptera. This suggests that these bats use different micro-habitats within the habitat where they co-occur.

## Introduction

Understanding species' interactions is a fundamental research area in ecology (Rickleffs & Schluter 1993) and these interactions (e.g., predation, competition) are frequently sited as causal factors in adaptive speciation (Dieckmann *et al.* 2004). This theory implies that each species is adapted to a specific ecological niche, separated from other species by reproductive isolation and eco-morphological traits (Hutchinson 1957; Mayr 1986; Schluter 2001; Holt 2009) which permit co-existence. Ecological interactions and selective processes contribute to the evolution of new phenotypes and the maintenance of morphological diversity (Dieckmann & Doebeli 1999; Ryan *et al.* 2007).

In many vertebrates species radiation and diversification of ecological niches is accompanied by corresponding diversification of morphological characters and adaptations. Case studies, e.g. Darwin's finches, have shown that morphological traits can be formed through natural selection interacting with trophic resources (Grant 1985; Schluter *et al.* 1985; Grant & Grant, 2006). Often these morphological traits are directly related to the performance of a species during resource exploitation and the ability to sustain that performance in a changing environment (Lack 1974; Schluter *et al.* 1985; Herrel *et al.* 2005). High diversity in such adaptations and variable resource exploitation in time and space may facilitate the coexistence of even highly similar species (Coyne & Orr 2004).

Bats (Chiroptera) exploit a great diversity of trophic niches with a variety of morphological and behavioural adaptations, but up to 70% are primarily insectivorous (Simmons 2005). Due to high species richness and diversity in trophic adaptations, bat communities and guilds have been the focus of numerous studies dealing with species interaction and community structure (Findley & Black 1983; Dumont 1997; Schnitzler & Kalko 2001; Aguirre *et al.* 2002; Kalko *et al.* 2007; Clare *et al.* 2009; Bohmann et la. 2011; Razgour, *et al.* 2011, Emrich et al. accepted).

To understand the interactions between species and to measure their ecological niche one can observe competitive interactions directly, but for cryptic and elusive species like bats, indirect measures are necessary including analysis of both morphological traits and behavioural mechanisms of resource exploitation. Echolocation call structure has been shown to separate ecological niches of bat species (Schnitzler & Kalko 2001; Schnitzler *et al.* 2003; Siemers & Schnitzler 2004). Similarly, wing morphology and corresponding flight habits and foraging behaviour are highly diverse and contribute to niche segregation (Norberg & Rayner 1987). Bite force also influences resource partitioning on the base of food hardness and prey handling (Freeman 1981; Dumont 1999, Aguirre *et al.* 2003; Santana *et al.* 2010). Often a complex of interacting parameters, including subtle traits such as temporal partitioning of resources (e.g. Emrich et al. accepted), must be considered to accurately measure the mechanisms of partitioning. These parameters partly define ecological niches (habitat, foraging, etc) and shape bat communities.

One method of assessing whether these characters effectively result in niche partitioning and specialization is to measure the effect on food resource exploitation and examine post foraging resource divisions. For example, through analysis of eco-morphological characters in conjunction with dietary analysis and modelling of niche differentiation between potentially competing species (Emrich et al. accepted). Traditionally, dietary studies have employed morphological identification of prey remains in faecal samples or stomach content (Whitaker 1972; Kunz & Whitaker 1983; Whitaker *et al.* 2009), or culled prey remains (Bell 1982; Jones 1990; Lacki & Ladeur 2001) to assess prey occurrences and dietary biomass. While effective, these methods are normally limited to ordinal or family level identification of prey and may overlook more subtle niche differentiation. More recently, molecular based approaches were introduced and have become sophisticated both in efficiency and productivity (Symondson 2002; King *et al.* 2008; Pompanon *et al.* 2012). Molecular methods provide the possibility of species-level taxonomic assignment of

unknowns (Hebert *et al.* 2003a, b) and rapid analyses particularly using high-throughput sequencing platforms, such as electronic-current based Ion Torrent (Pourmand *et al.* 2006; Rothenburg *et al.* 2011; Pompanon *et al.* 2012). These methods have been used to great effect in insectivorous bats with both traditional sanger sequencing (Clare et al. 2009, 2011, Zeale et al. 2011) and high throughput next generation sequencing (Bohman et al. 2011, Razgour et al. 2011, Clare et al. a/b accepted, Emrich et al. accepted, Krüger et al. accepted).

Two species of trawling *Myotis*, *Myotis dasycneme* and *Myotis daubentonii,* share behavioural and morphological traits such as large feet, foraging close to the water surface, and scooping prey from the surface with their feet or tail membrane. During foraging both species use short, downward-frequency-modulated echolocation signals, of 1.7–3.0 ms length and a sweep range of 38.9–54.5 kHz (Jones & Rayner 1988; Kalko & Schnitzler 1989; Britton *et al.* 1997; Siemers *et al.* 2001). A previous study based only on morphological diet analysis showed high overlap in prey groups, with little difference among the less frequent prey items (Krüger *et al.* 2012). In contrast, these species show dissimilarities in roosting behaviour and migration behaviour. *M. dasycneme* prefers synanthropic roosting, using attics and cavity walls as maternity roosts while *M. daubentonii* is frequently found in hollow trees and artificial roosts in forests. In addition, it is believed that they do not share a recent phylogenetic history thus resource competition may not have been a primary factor in their radiation. *M. dasycneme* probably diverged more than 10 MYA from a group of *Myotis*, which includes *M. daubentonii*. This leaves *M. dasycneme* more closely related to *M. mystacinus*.Yet, the phylogenetic position of *M. dasycneme* is debated (Stadelmann *et al.* 2004, 2007).

Razgour *et al.* (2011) assessed resource use between cryptic, closely related long-eared bats (*Plecotus auritus* and *P. austriacus*) occurring in sympatry while Bohman *et al.* (2011) considered resource use between two morphologically different species which share roosts and foraging grounds (*Chaerephon pumilus* and *Mops condylurus*). Here we consider the

115 intermediate case, two sympatric species of the same guild which do not share a sister-species
116 relationship. While resource partitioning in general may be important in diversification and
117 coexistence, morphological convergence and the limits of bats' perceptual abilities may limit
118 prey partitioning. In this scenario, morphology and echolocation may lead to habitat selection
119 and thus dietary convergence, particularly in insects are not limiting and thus competition
120 unlikely. Here, we test the hypothesis that morphological and behavioural convergence
121 corresponds with resource overlap when there is no reasonable expectation of past radiation
122 via competitive interactions (e.g. allopatric origin, reproductive isolation).

123 We use morphological traits, including wing morphology, bite force and a novel
124 aspect, the physio-morphological ability to lift objects from the surface of the water (and thus
125 important for trawling bats), along with molecular and morphological dietary analysis, to
126 assess mechanisms of co-existence between these two predators. We hypothesise that the
127 dietary niche of the two bat species will overlap to a large extent, as both species should
128 perceive similar sized prey and use similar hunting modes in the same habitats. Their physio-
129 morphological abilities, though similar (same guild), may vary reflecting more recent
130 competitive interactions in secondary sympatry. We expect that both species share major prey
131 types and do not show significant eco-morphological differences.
132

## Methods

The study uses a combined approach including data collected from molecular and morphological analysis of diet from faecal pellets, measurements of wing morphology, bite force and laboratory experiments on hunting performance.

*Study site and guano collection*

We collected faecal samples between May and August 2009 from bats mist-netted along their commuting routes between roosts and foraging habitat over the Schwentine River in Schleswig-Holstein, Germany (54,195°N; 10,308°E). The distances between the sampling sites varied from 2.94 km to 14.61 km. Thus given the proximity and similarity of the landscape at each site, we consider them to be "sympatric" (able to commute freely between sites) and that any observed difference in diet between the species is unlikely to be explained through access to different species of insects via habitat selection. We kept bats in clean soft cotton bags for approximately half an hour after capture for collection of faecal samples. Permission and ethical approval was provided by the State Agency for Agriculture, Environment and Rural Areas, Schleswig-Holstein, Germany (LANU 314/5327.74.1.6).

*Functional morphology*

Wing morphology — We photographed wings and measured wing parameters of 30 bats with the program AxioVision 4.7.10 (Carl Zeiss Microscopy GmbH, Jena) along with collection of traditional morphological data, like body mass and forearm length (Norberg & Rayner 1987).

Weight lifting — To estimate weight lifting in foraging performance we took six male *M. daubentonii* and three male *M. dasycneme* into captivity for laboratory experiments. We

157 collected data in the laboratory facilities at the Max-Planck Institute for Ornithology,
158 Seewiesen, Germany. We housed these animals in air-conditioned rooms (20°C / 80%
159 humidity) with *ad libitum* water and food supply (mealworm larvae, *Tenebrio molitor*,
160 vitamins and minerals in addition). Animals were habituated to a 12 h shift in their
161 photoperiod. Permission and ethical approval was provided by the State Agency for
162 Agriculture, Environment and Rural Areas (LLUR), Schleswig-Holstein, Germany (LLUR
163 515/5327.74.1.6).

164 We trained bats to take mealworms from the water surface of an artificial pond (3 × 4
165 m), built in a 4 × 9 meter flight room. For measurements of maximal weight lifting
166 performance we connected a dummy mealworm with a piezo electric force transducer (type
167 5015A, KISTLER, Inc.) via a nylon thread and a custom made deviating mechanism. The
168 dummy was connected permanently with the nylon thread so that maximum lift force could be
169 obtained. After each catching attempt (successful or unsuccessful) a real mealworm was
170 provided on the water surface.

171 Bite force — All bats caught in the field were identified, sexed, weighed, and
172 measured. We only choose adult bats for bite force assessment. Measurements included
173 forearm length (the standard proxy for bat body size) and upper tooth row length (distance
174 from the canine to the 3$^{rd}$ molar, CM$^3$), used as a proxy for head size. We measured maximum
175 bite force in 20 bats each of *M. dasycneme* and *M. daubentonii*, by letting the bats bite onto a
176 custom-made lever which connected to a piezo electric force transducer (KISTLER, Type
177 9217A) (Aguirre *et al.* 2002). The distance of the bite plates was adjusted to accommodate a
178 standardized gape angle of approximately 25°(Dumont and Herrel 2003). A series of six bite
179 sessions was conducted, some sessions consisting of multiple bites. The maximum bite force
180 obtained across all bite sessions was used for further analysis. Bite forces were corrected for
181 the effect of the lever and transducer system. We released bats directly after measurements at
182 the site of capture.

*Molecular diet analysis*

We extracted DNA from each pellet (n *M. dasycneme* = 34; n *M. daubentonii* = 36) using the QIAamp DNA Stool Mini Kit (Qiagen, UK) following standard protocol with adjustments suggested by Zeale *et al.* (2011). We stored extracted DNA at -80°C prior to PCR analyses. We amplified insect DNA from faecal pellets using insect general COI primers ZBJ-ArtF1c and ZBJ-ArtR2c modified as described by Clare et al. accepted. The original primers were described by Zeale et al. (2011), and have been tested by many recent studies (e.g. Bomann et al. 2011, Razgour *et al.* 2011, Clare et al. a/b accepted, Emrich et al. accepted). The target region is a 157 bp amplicon located at the 5' end of the 658 bp COI barcode region (Hebert *et al.* 2004). Prior to experimental use we confirmed the efficiency of the primers on additional common local arthropod genera (i.e. Diptera, Aranea, Lepidoptera, Coleoptera, data not shown) by amplification following Zeale et al. (2011). We did not use unique MID recognition methods (e.g. Clare et al. a accepted), rather, all independently amplified samples were pooled within predator species (following Emrich et al. accepted) for DNA sequencing via the Ion Torrent sequencing platform (Life Technology) at the University of Bristol Genomics facility (School of Biological Sciences, Bristol, UK). To remove primers and adaptors post sequencing, collapse to unique haplotypes and for further sequencing processing, we used the Galaxy V platform (https://main.g2.bx.psu.edu/root; Giardine *et al.* 2005; Blankenberg *et al.* 2007; Blankenberg *et al.* 2010; Goecks *et al.* 2010). We removed haplotypes represented by <2 haplotypes and clustered the sequences into molecular operational taxonomic units (MOTU) using the program jMOTU (Jones *et al.* 2011). We tested grouping thresholds from 1-10 bp and selected a 4bp threshold for this data set (see Razgour *et al.* 2011). We extracted representative sequences for each MOTU for comparison with a known reference library.

We compared sequences against known reference sequences within the Barcode of Life Data Systems (Ratnasingham & Hebert 2007; Clare *et al.* 2009). If sequences matched completely to a reference sequence without matching any other arthropod, we regarded the sequence as belonging to the same species. However, the short amplicon length also constrains some species identifications. Following Clare et al. a.b accepted we used a modified version of the criteria in Razgour *et al.* (2011) as follows:

1a. True species match (>99 % similarity)

1b. True species match (>98% similarity)

2. Match (>98%) to more than one species, only one of which belongs to local assemblage

3. Match (>98%) to several species or genera – genus or family level assignment made and considered provisional.

*Morphological diet analysis*

For morphological faecal analysis, we dried guano samples (n = 206) at room temperature and stored them at -20°C to avoid coprophagous insects. Before analysis, pellets were soaked for 48 h in 70% Ethanol and dissected under a binocular microscope (×40 – 60). Characteristic fragments were separated and mounted in Euparal for further examination. We identified prey groups by fragments to class, order, family, or genus level (where feasible), by comparison of fragments with whole collected insects and arthropod identification keys (McAney *et al.* 1991, Krüger *et al.* 2012).

For each individual bat, we calculated the occurrence of each prey group as the relative proportion of all sampled individual bats (N) ('percentage occurrence', total > 100 %). We further determined the relative proportion for each prey group of the total of

consumed prey groups (Nc) ('percentage frequency', total = 100) (McAney *et al.*1991; Vaughaun 1997; Krüger *et al.* 2012).

*Data analysis*

We assessed differences in functional-morphological traits (e.g., wing morphology, bite force and lifting performance) using R (R Development Core Team 2009, Version 2.15.1). To estimate niche differences between *M. dasycneme* and *M. daubentonii* based on the molecular dietary data we calculated Hamming distance and Bray-Curtis index for similarity. The Hamming distance gives the number of positions at which the corresponding symbols of two strings of the same length are different (Hamming 1950). It is calculated on the entire pool of available prey. A smaller value for Hamming distances indicates more similar dietary choices and includes shared prey and shared avoidance of prey in the similarity score. The Bray-Curtis index (Equation 1) (Bray & Curtis 1957) is used to quantify the dissimilarity in the dietary composition of the study species, where $C_{ij}$ is the sum of the lesser value for only those species in common between both samples. $S_i$ and $S_j$ are the total number of species counted in both samples. The Bray–Curtis dissimilarity is 0, if the two samples share all species and 1, if the two samples do not share any species (Bloom 1981).

$$BC_{ij} = \frac{2C_{ij}}{S_i + S_j} \qquad \text{(Equation 1)}$$

$$D = 1 - \sum_{i=1}^{s} \frac{n_i\,(n_i - 1)}{n\,(n-1)} \qquad \text{(Equation 2)}$$

$$O_{jk} = \frac{\sum p_{ij} p_{ik}}{(\sum p_{ij}^2 \sum p_{ik}^2)^{1/2}} \qquad \text{(Equation 3)}$$

To assess dietary niche breadth based on the morphological diet data, we used the Simpson's index for diversity and heterogeneity (Equation 2), where $n_i$ is the relative proportion of a prey item *i (*with $i = 1…n$) of a total of *n* prey items. Thus, D is 0, if all eaten prey belongs to one prey group. The higher the diversity, the closer D gets to 1. To estimate the degree of similarity in prey exploitation based on the presence-absence data, we calculated Pianka's index of niche overlap (Equation 3), where $p_i$ is the frequency of occurrence of prey item *i* in the diet of species *j* and *k* (Pianka 1973). The Pianka's index reaches 1, if diets of *j* and *k* overlap to a 100%. To test the effect of species or sex on the variance in the dietary data we conducted a permutation analysis of variance (ADONIS, Anderson 2001). Additionally, we performed non-metric multidimensional scale ordination (NMDS) with Jaccard distance to visualise differences between the two species (Clark & Warwick, 2001). We tested differences in single prey groups, also including the prey habitat, between species with generalised linear models (GLM) and Tukey post-hoc tests.

We estimated species richness and diversity using morphological dietary data with the *vegan* library (Oksanen *et al.* 2011). We conducted multivariate methods, NMDS, Adonis and GLM, using the *vegan* R library (Oksanen *et al.* 2011) and the *MASS* R library (Venables & Ripley 2002).

## Results

### *Functional morphology*

We measured wing parameters from 30 bats using digital photos of live animals (Table 1). The two species differed significantly in their basic body measures: body mass ($\chi^2$ = 21.08, df = 1, p < 0.001) and forearm length ($\chi^2$ = 18.73, df = 1, p < 0.001). Within species we found differences, with females being larger in *M. daubentonii* and males being larger in *M. dasycneme*. The species differed in wingtip shape index (I) (t = 2.0739, df = 27, p < 0.05), but not in wing loading (t = 1.3785, df = 27, p = 0.179). Yet, these parameters show high variability within and between species when taking the sex into account: Male *M. dasycneme* showed higher I than male *M. daubentonii*, vice versa for female bats (Tab 1). We measured weightlifting performance in seven male *M. daubentonii* and three male *M. dasycneme*, each represented by 10 individual measurements, under the same settings and conditions. The two species differed significantly in maximal weight lifting performance (t = -7.08, df = 8, p < 0.001). We found *M. dasycneme* individuals to perform less well than *M. daubentonii*. The Pearson correlation shows that wing loading and weightlifting performance are negatively correlated (cor = -0.83, p<0.01, Fig. 1), though this is not significant in *M. daubentonii*, when tested separately.

The values for maximal bite force differed significantly between species (t = 8.68, df = 37, p < 0.001). We found *M. dasycneme* to have higher maximal bite force congruent with a longer upper tooth row length ($CM^3$) (Bite force = 31 N; $CM^3$ = 6.12mm, sd = 0.21) than *M. daubentonii* (Bite force = 19 N; $CM^3$ = 5.2mm, sd = 0.23). In addition, we correlated the maximal bite force with mean forearm length (FA), which is a proxy for body size, and mean upper tooth row length ($CM^3$), which indicates head size. Both size parameters correlated positively with maximal bite force when tested in all species (Fig. 2) though if tested

297 separately, only *M. dasycneme* shows positive correlation between tooth row length ($CM^3$)
298 and maximal bite force (rho = 0.51, $p < 0.05$).
299
300

*Molecular diet analysis*

We identified a total of 176 MOTUs, of which 125 could be assigned to insect taxa. For 51 MOTUs we found no matches in the BOLD Systems. We rejected 3 MOTUs, either because they were too short or because they matched unrelated taxa (e.g. Fungus). We found 135 MOTUs in samples from *Myotis daubentonii*, whereas 77 MOTUs were assigned to samples from *Myotis dasycneme*.

We found high values for Bray-Curtis index (BC) between *M. dasycneme* and *M. daubentonii* (Table 3). However, there are gender specific differences. Females show lowest similarity between species. Similarly, there is a high Hamming Distance between *M. daubentonii* females and *M. dasycneme* females (Table 3). We found lower distances within *M. dasycneme*, between males of both species and between *M. daubentonii* males and *M. dasycneme* females. Overall dietary divergence as measured by Hamming Distance between *M. dasycneme* and *M. daubentonii* was higher than similar comparisons within species (Table 3).

Within the identified prey species (n = 51), some specific prey habitat interactions are apparent. The Lepidoptera we found in the samples from *M. dasycneme* encompasses three species, which either have aquatic life stages (*Acentria ephemerella*) or develop in close proximity to aquatic ecosystems (*Nonagria typhae, Leucania obsoleta*). Other species like *Epinotia demarniana* or *Mompha epilobiella* are known from riverine habitats with larvae feeding on riverine plant species (e.g., *Alnus glutinosa*, *Epilobium sp.*). The prey species in the order of Hemiptera clearly indicate aquatic habitats, as all found species show sub-aquatic life cycles, with occasional flight events (e.g., *Sigara striata*). Beetles, assigned to truly terrestrial species (*Copris sp.* and Carabidae), were only consumed by *M. daubentonii*.

*Morphological diet analysis*

Overall, we analysed 206 samples of *M. dasycneme* (n = 84) and *M. daubentonii* (n = 122). In the diet of *M. dasycneme* we identified 12 prey groups and for *M. daubentonii* 17 prey groups. Within identified Diptera, we could identify the sub-order Nematocera with the families of Tipulidae and Chironomidae and the genus *Glyptotendipes*, and the sub-order Brachycera. Within the Hemiptera, we were able to identify the families Corxidae, Gerridae and Aphidoidea. The two predators showed high dietary overlap and similar niche breadth.

The ADONIS analysis indicated significant differences in the diet of the two species (ADONIS: $F = 2.53$, $P < 0.05$). The NMDS ordination resulted in a two-dimensional solution with a final stress of 0.132. Samples of *M. dasycneme* and *M. daubentonii* are evenly spread out in the diagram and overlap strongly (Fig. 3).

The Simpson's index showed no statistically significant differences between species in diet breadth or the diversity of prey taxa (*M. dasycneme*: 0.75; *M. daubentonii*: 0.82; $\chi^2 = 90.3281$, df = 1, $p < 0.001$). Additionally, Pianka's index for niche overlap indicated an overlap of nearly 100% (Table 5). Comparing the single prey groups between the species' diets, only chironomids differed significantly between the two bat species (Table 5). Unknown Diptera and Brachycera also occurred, but not significantly more often in the diet of *M. daubentonii*. Similar observations concern chironomid pupae in the diet of *M. dasycneme* (Table 5). Both species displayed differences in prey occurrence regarding the major habitat where prey groups are found (GLM, aquatic: $z = -0.009$, $P < 0.05$; terrestrial: $z = 0.902$, $P = 0.367$).

## Discussion

We test whether morphological and habitat convergence correlates with dietary overlap and we assess the potential for micro-niche differentiation in morphological and behavioural characteristics. Our analysis suggests that these two bat species overlap largely in both in morphological features and diet but may demonstrate minor differentiation based on behaviour and micro-habitat selection. We provide a multi-factor analysis of the trophic interactions between two morphologically similar species that lack a recent phylogenetic divergence.

### *Functional morphology*

Flight modes and behaviour vary among flying animals. Bats show great diversity in wing morphology and flight patterns (Findley 1972; Norberg & Rayner 1987), triggered by adaptive processes in response to resource availability e.g., prey exploitation and habitat utilization. In bats, wing morphology has been used to identify and characterise structures of communities, guilds and assemblages (Findley 1972; Norberg & Rayner 1987; Britton *et al.* 1997). Our results support the classification of *M. dasycneme* and *M. daubentonii* as trawling *Myotis*, of the Leuconoe guild (Findley 1972; Baagøe 1987; Norberg & Rayner 1987). *Myotis dasycneme* and *M. daubentonii* both show adaptations like lower wing loading, compared to fast flying species like *Nyctalus noctula*, which allow relatively slow flight above water surfaces. Both bat species show high similarity in wing morphology, which, together with high similarity in echolocation (Siemers *et al.* 2001), implies that both bat species perceive and exploit the same prey when they are in the same habitat. We found wingtip shape (I) to be highly variable within the species (female-male difference). Still the higher wingtip shape index (I) in *M. daubentonii* might indicate better maneuverability. *M. daubentonii* is known, to utilizes heterogeneous foraging habitats, like riverine forests, river banks and lake shores,

but also occurs and hunts within forests and cluttered backgrounds (Taake 1992, Dietz *et al.* 2010, Nissen *et al.* 2013). For *M. dasycneme,* less is known about habitat preferences though they are thought to hunt primarily over and along large water bodies (e.g. lakes, canals, rivers) (Limpens 2001), but other, more structured habitats like reeds and forest edges are also used (pers. observation).

The variance in wing parameters found within species may be explained by adaptive radiation following competition. Many insectivorous bat species exhibit sexual segregation regarding habitat differences. Different morphological adaptations would facilitate different habitat utilization. For example, male and female particoloured bats (*Vespertilio murinus*) use different foraging habitats (Safi et al. 2007), as do barbastell bats (*Barbastellus barbastella*) (Hill *et al.* 2011). Within *M. daubentonii* females and males may utilize different habitats and even regions (Dietz *et al.* 2009). In *M. dasycneme* it has been observed that female and male individuals inhabit different regions with different habitat interior in the Netherlands (A-J. Haarsma, pers. comm.).

The ability to carry higher load is correlated with behaviour. The ghost bat, *Macrodermas gigas* (0.12 kg), can carry up to 60 g (= 50% of its own weight), which allows it to sustain a diet of small mammals (Kulzer *et al.* 1984). The vampire bat *Desmodus rotundus* can take up 100% of its own weight in blood, also a necessary adaptation, which allows this species to maintain a nutritionally low blood diet (Wimsatt 1969). Fruit bats regularly carry heavy fruits and seeds, like avocado or mangoes (Marshall 1983; Richards 1990). *Myotis capaccinii*, also a trawling *Myotis* and facultative piscivore, is able to carry 0.5g fish (Aihartza *et al.* 2008). In all, lift capacity may be a fundamental character in niche specialization in bats thus the subtle differences measured here are intriguing. However, these measurements should be treated cautiously. Although these same flight room parameters have been successfully used previously with these species (Siemers *et al.* 2001), the difference we found in weightlifting performance might be partially explained by the aerodynamic

399 constraints pond bats had to face in the flight room. Due to kinetic laws, *M. dasycneme* would
400 likely reach a higher weightlifting capacity with higher flight speed (F = m * a). Indeed,
401 higher speeds have been observed in the wild (Baagøe 1987) and are apparent in the square
402 root of their wing loading, which is proportional to flight speed (Norberg & Rayner 1987).
403 Despite these potentially subtle differences, *Myotis dasycneme* and *M. daubentonii* can be
404 regarded as similar in morphological terms, hence the same guild and sub-genus.

405 The results for bite force show some differences between the species. Although both
406 are insectivorous and feed mainly on soft bodied prey (e.g. Diptera, Lepidoptera), *M.*
407 *dasycneme* had a higher bite force than *M. daubentonii*. These differences result from the
408 overall size differences between the species, particularly head- and jaw length, head width and
409 resulting jaw muscle size (Herrel *et al.* 2001; Aguirre *et al.* 2002; Herrel *et al.* 2005) which
410 are larger in *M. dasycneme*. Both species lie well within the variation range in bite force and
411 size measurements for their family Vespertilionidae (Greif *et al.* unpublished). This
412 morphological distinction cannot be fully explained by the prey. On the one hand the bats
413 show subtle differences in consumed prey size. Moths of larger wingspan (>20mm), like
414 *Apamea monoglypha, Nonagria typhae* or *Laspeyra flexula*, appear only in guano samples of
415 *M. dasycneme*. A bigger mouth may lead to a more efficient handling of bigger prey items
416 (Herrel *et al.* 2005). On the other hand, both bats prey on beetles, as well as other hard bodied
417 prey like water boatman (Corxidiae). Although the molecular diet data only show beetles
418 (Carabidae) to occur in the diet of *M. daubentonii,* the morphological results show no
419 difference in beetle consumption between the two species. Hence, bite force needs to be
420 discussed cautiously as meaningful trait within niche differentiation of *M. dasycneme* and *M.*
421 *daubentonii*. The major prey items (Diptera, Lepidotpera) are all soft-bodied prey.

422 One limitation of our morphological and behavioural data was a limited sample size.
423 The conservation situation for both species limited the number of individuals that we may
424 take into captivity. To compensate we have performed a repeated measures design and

425 analysis but the conclusions drawn must be considered preliminary in light of the small
426 sample.
427
428 *Dietary Analysis*
429 As predicted, the dietary niches overlap to a high degree between species, which
430 mirrors the morphological and behavioural similarities. In particular, both *M. dasycneme* and
431 *M. daubentonii* feed to a large extent on Diptera and Trichoptera. Although, the niche breadth
432 differed between the species, the morphological dietary data overlapped nearly 100%. *M.*
433 *daubentonii* seems to exploit a larger variety of prey compared to *M. dasycneme*, which seems
434 to rely on chironomids to a larger extent. The comparison of prey regarding their major
435 habitats, shows that *M. dasycneme* overall depends more on the aquatic prey fauna and less on
436 the terrestrial, contrasting slightly with *M. daubentonii*. The molecular data indicates that
437 females may be particularly different between species. Females have higher energy demands
438 and nutrition requirements during pregnancy and lactation. This is due to a reduction in time
439 spent torpid and to promote growth and development of the foetus (Swift & Racey 1983;
440 Wilde *et al.* 1995, 1999). To compensate for this increase in total energy demand, female bats
441 need to increase food consumption (Anthony & Kunz 1977; Kurta et al. 1989; Kunz et al.
442 1995; Racey & Entwistle 2000; Encarnacao & Dietz 2006). Often they are found to forage in
443 areas with higher insect abundance compared to males (Dietz *et al.* 2006). In our data the
444 higher energy demand of females may translate into the broader niche breadth compared to
445 males, because generalistic feeding behaviour may provide their optimal foraging strategy
446 (Stephen & Krebs 1986). In this context, the higher dietary distance between females of the
447 different species appears reasonable. If females choose to forage in patches with high food
448 supply within aquatic habitats, they are more likely to meet and compete for food resources.

449 Consequently, the greater distance between females may be a result of a mechanism to avoid
450 such competition.

451 The molecular results show high resolution in prey identification and exceed the
452 number of identified prey found through morphological analysis. Molecular analysis is
453 particularly powerful for the identification of small morphologically cryptic prey such as
454 chironomid species. With the morphological tools we could only identify one genus
455 (*Glyptotendipes*, Chironomidae) within this prey group. But the molecular approach revealed
456 and estimated 11 species, though this is still small compared to the actual number of
457 chironomid species which can be expected in the central Europe (e.g. ~ 700 species are found
458 in Germany). The highly diverse group of Chironomidae harbour many cryptic species and
459 are morphological hard to distinguish (Cranston 1995) leading to a significant taxonomic
460 ambiguity in both morphological and molecular reference collections. A lack of species
461 sequences in the barcode archives certainly constrains output in molecular data.

462 While molecular analysis is becoming common within dietary studies because of its
463 significant taxonomic resolution, there are key advantages of traditional morphological
464 analysis. For example, we were able to distinguish different life stages of prey groups, like the
465 pupal form of Chironomidae. This can provide very valuable information on the hunting
466 mode of the focal species, in this case true trawling behaviour, when the bat scoops the not yet
467 fully emerged Chironomid together with the pupal case directly from the water surface. It can
468 also indicate foraging areas, like the pelagic areas of lakes, where Chironomidae undergo
469 mass emergences. There are clearly advantages of pairing molecular and morphological data
470 for measuring niche differentiation.

471 The abundance of prey species in the foraging habitats is high. For example, many of
472 the Lepidoptera species are highly numerous and abundant during their adult stage (*Idea*
473 *biselata, Acentria ephemerella, Mompha epilobiella*). Also Diptera (Nematocera, like
474 Chironomidae), Trichoptera and especially Ephemeroptera are known to be numerous and

abundant in water habitats (Ward 1992; Racey *et al.* 1998; Warren *et al.* 2000). Hence, our results reflect the diet of generalist predators in this particular habitat. Both, the morphological and the molecular data, suggest the bats share major prey groups like Diptera, Trichoptera and Lepidoptera.

The phylogenetic position of *M. dasycneme* within the old world *Myotis* bats is still disputed (Ruedi & Mayer 2001; Stadelmann *et al.* 2007; Jiang *et al.* 2010). But regardless of this ambiguity, all agree that *Myotis dasycneme* and *M. daubentonii* do not to share a recent phylogenetic history and likely evolved in allopatry and thus without competition. It is thought that *M. dasycneme* is genetically situated more close to *M. mystacinus*, where as *M. daubentonii* belongs to a group of *M. nathalinae* and *M. bechsteinii*. The geographical origins are unknown (Stadelmann *et al.* 2007). Additionally early studies have shown that morphological similarities rarely reflect close phylogenetic relationships, which is illustrated by the close phylogenetic relation of the ecologically and morphological different *M. daubentonii* and *M. bechsteinii* (Ruedi & Mayer 2001).

*Resource partitioning and mechanisms of species coexistence*

Our data confirm that these species show high morphological and behavioural convergence which leads directly to high trophic overlap. But we also distinguish subtle but significant differences in bite force and lift force which corresponds to small differences in predator body size and explains subtle differences in prey exploitation.

Partitioning of resources and micro-resource differentiation is leading hypothesis to explain the coexistence of species and radiations. Emrich et al. (accepted) explored the resource use by an ensemble of Jamaican bats and found that a variety of behavioural and morphological characters contribute to patterns of resource use including things as subtle as temporal partitioning of hunting grounds. The hypothesis that resources must be partitioned rests on the assumption that some aspect of the resources is limited and thus limiting leading

to competition. We have found little evidence of partitioning of insect resources here and, in fact, there is very little evidence to suggest that insects are a limited resource in general. Thus competition for this resource may be minimal among sympatric bats. The alternative hypothesis is that habitat selection is based on morphological and perceptual abilities and thus similar habitat selection by bats with similar echolocation should result in a high degree of dietary overlap. This is largely what we have observed here.

In our analysis, we noted subtle differences in the dietary profile of these bats. While these are real, it is particularly interesting to consider whether these differences are biologically meaningful. First, it is important to note that while morphological data is limited in its ability to recognize subtle differences, molecular data, which identified prey at the species level, is likely biased towards the detection of resource partitioning. This method will tend to overrepresented rare items and underestimate the importance of common items (Clare et al. a/b accepted). As such, it is almost certain that two dietary analyses will contain species that are different (as we have seen here). To differentiate these random differences from biologically meaningful partitioning, we must consider whether the bats can differentiate at this level. While low duty-cycle bats very likely perceive insects by size, shape, speed and acoustic reflectivity, it is unlikely that they differentiate subtle morphological differences between species. As such, we must treat minor species-level differences conservatively. Of particular interested in our analysis are aspects which suggest a significant behavioral difference, for example, we observed that *M. dasycneme* was almost twice as likely to consume Chironomid pupae and more likely to consume prey with aquatic habitats. This suggest a difference in hunting style which may be a far more significant form of micro-resource partitioning that any particular species-level difference in diet. As such, strict differences should be considered in light of their relevance to behaviour. The power of these analyses will be seen when these high-resolution dietary analyses are used to test specific behavioural hypotheses and to guide perceptual test of bats' echolocation ability.

## Conclusions

By selective adaptation of morphological and sensory features, evolution permits a species to improve its capacity to use certain food resources in distinct ways and thus shapes communities of foraging bats. Both bat species show high overlap in their functional morphology and also in their diets. Yet, we cannot overlook the dietary differences found between the two species suggest behavioural differences in hunting style. Our study strongly advocates that the integration of different methodologies is crucial to address characteristics of ecological niches and species interactions.

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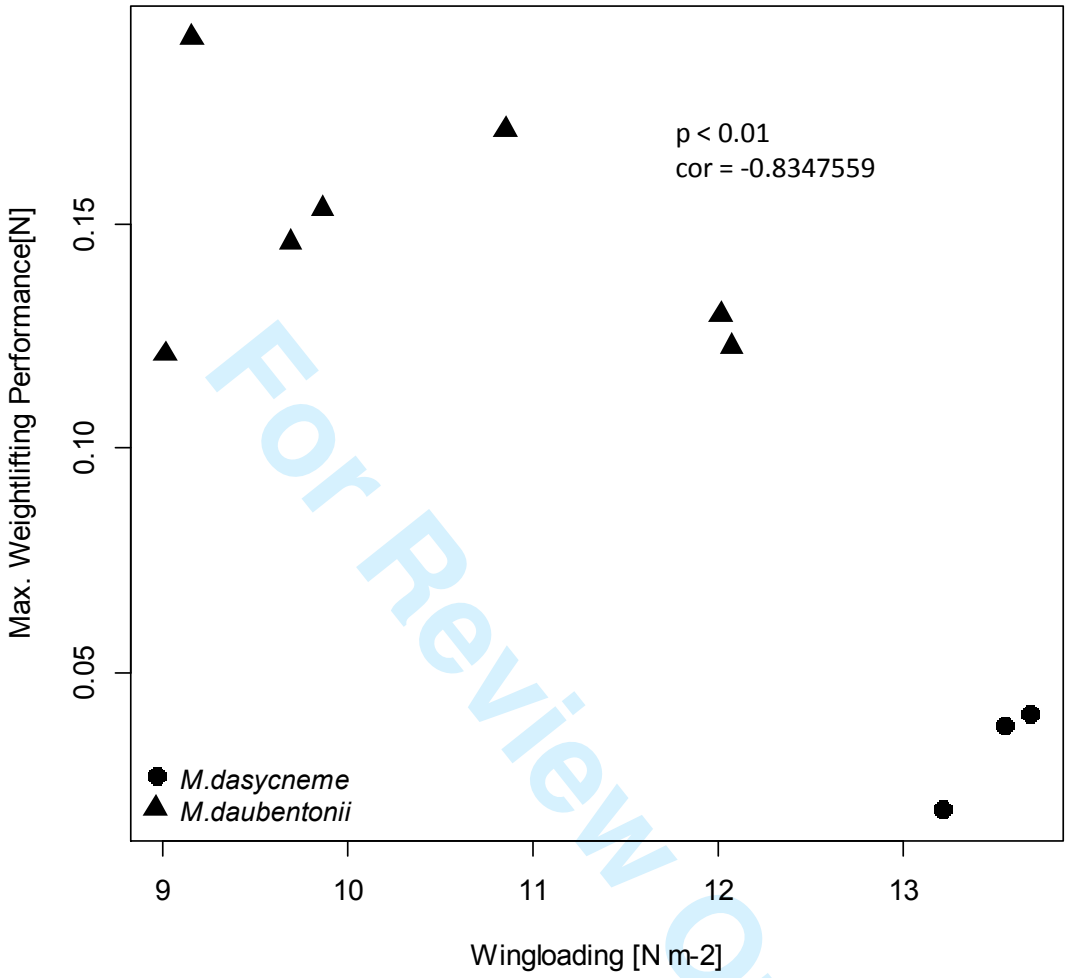




**Fig. 1 Plot of maximal weightlifting performance (N) against wing loading [$Nm^{-2}$] in *M. dasycneme* and *M. daubentonii*. Each point represents the max value of ten measurements under the same conditions and settings. Additionally the Pearson correlation of maximal measured weightlifting performance and wing loading shows a strong negative correlation.**

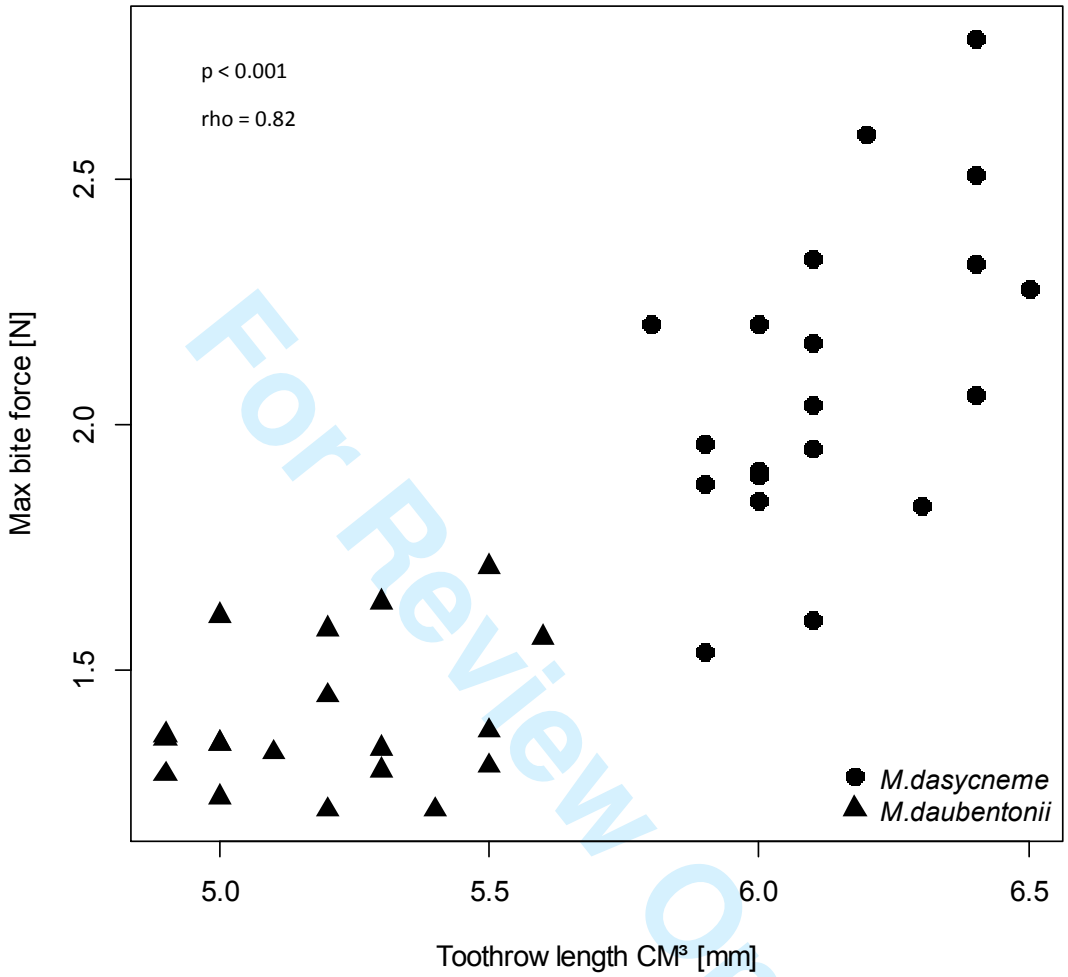




**Fig. 2 Plot of the maximal measured bite force against the upper toothrow length (CM³) from *M. dasycneme* (circles) and *M. daubentonii* (triangles). Additionally the result of Spearman rank correlation of these two parameters is given, indicating a significant positive correlation.**

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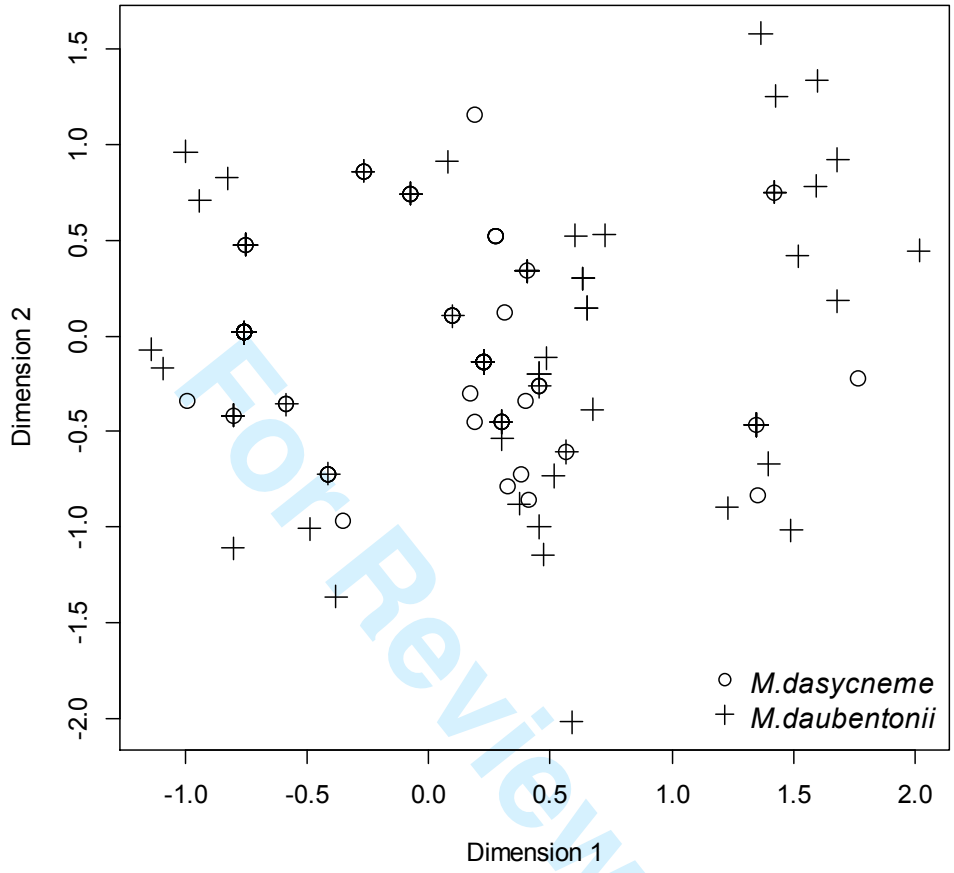


**Fig. 3 Plot of a non-metric two-dimensional ordination scale (NMDS) based on the presence-absence prey data derived from the morphological diet analysis on M. dasycneme (circle) and M. daubentonii (corss) (n= 206, stress = 0.132).**

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**Table 1 Values of body mass and forearm length taken from live bats and wing morphology measurements, taken from pictures, for *M. dasycneme* and *M. daubentonii* (mean ± SD).**

| | *Myotis dasycneme* | | *Myotis daubentonii* | |
|---|---|---|---|---|
| Variable | Male | Female | Male | Female |
| | n = 10 | n = 4 | n = 11 | n = 5 |
| Body mass | 17.51 ± 0.6 | 16.95 ± 0.3 | 10.125 ± 0.6 | 12.12 ± 1.3 |
| Forearm length | 4.687 ± 0.036 | 4.6725 ± 0.008 | 3.754 ± 0.03 | 4.095 ± 0.23 |
| Wing loading | 13.793 ± 0.739 | 11.86 ± 0.35 | 11.839 ± 0.68 | 13.08 ± 1.28 |
| Wingtip shape index | 1.746 ± 0.152 | 1.273 ± 0.141 | 1.135 ± 0.073 | 1.923 ± 0.412 |

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**Table 23 Taxa, identified in the diet of *Myoti dasycneme* (Mdas) and *M. daubentonii* (Mdau), which were assigned to MOTU utilising the BOLD search system ( V.3). The confidence levels (Conf) signify (1a) perfect match to one genus or species (>99%), (1b) match to one genus or species (>98%), (2) match to more than one species, of which only one was a local species, (3) match >98% to several species of different genera or to reference sequences only identified to family level. In the species columns (Mdas/Mdau) 1 stands for presence and 0 for absence of prey.**

| Order | Family | Species | Conf | Mdas | Mdau |
|---|---|---|---|---|---|
| Diptera | Anthomyiidae | *Delia florilega* | 1b | 0 | 0 |
| | Chaoboridae | unknown | 3 | 1 | 1 |
| | Chironomidae | *Chironomus sp.* | 3 | 1 | 1 |
| | | *Conchapelopia melanops* | 1b | 0 | 1 |
| | | *Cryptochironomus supplicans* | 1b | 1 | 1 |
| | | *Cryptochironomus sp.* | 1b | 0 | 1 |
| | | *Dicrotendipes tritomus* | 1b | 1 | 1 |
| | | *Microtendipes brevitarsis* | 1a | 1 | 1 |
| | | *Paracladopelma winnelli* | 1q | 0 | 1 |
| | | *Paratanytarsus tenuis* | 1b | 0 | 1 |
| | | *Procladius nigriventris* | 1a | 0 | 1 |
| | | *Procladius signatus* | 1b | 0 | 1 |
| | | *Procladius sp.* | 3 | 1 | 1 |
| | | *Tanytarsus brundini* | 1a | 0 | 1 |
| | | *Tanytarsus mendax* | 1a | 0 | 1 |
| | | *Xenochironomus xenolabis* | 1a | 0 | 1 |
| | | unknown | 1b | 0 | 1 |
| | Chloropidae | *Aedes sp.* | 3 | 1 | 0 |
| | Culicidae | *Anopheles sp.* | 1b | 1 | 1 |
| | | *Hilara quadrifasciata* | 1b | 0 | 0 |
| | Empididae | *Atophpthalmus inustus* | 3 | 1 | 0 |
| | Limoniidae | *Euphylidorea sp.* | 1a | 1 | 0 |
| | | *Helius flavus* | 1b | 0 | 1 |
| | | *Limnophila pictipennis* | 1a | 1 | 1 |
| | | *Limonia nubeculosa* | 1b | 1 | 1 |
| | | *Molophilus sp.* | 1a | 0 | 1 |
| | | *Pseudolimnophila lucorum* | 3 | 0 | 1 |
| | | unknown | 3 | 1 | 1 |
| | Pedicidae | unknown | 3 | 1 | 1 |
| | Psychodidae | *Simulium sp.* | 1b | 0 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| | Simuliidae | *Rachispoda lutosa* | 3 | 0 | 1 |
| | Sphaeroceridae | unknown | 3 | 0 | 1 |
| | Stratiomyidae | unknown | 3 | 1 | 0 |
| | Syrphidae | *Nephrotoma scalaris* | 1b | 0 | 1 |
| | Tachinidae | *Tipula scripta* | 1a | 0 | 1 |
| | Tipulidae | *Tipula sp.* | 3 | 0 | 1 |
| Lepidoptera | Coleophoridae | *Coelophora kuehnella* | 1a | 0 | 0 |
| | Crambidae | *Acentria ephemerella* | 1a | 1 | 1 |
| | | *Herpetogramma sp.* | 2 | 1 | 0 |
| | | *Scoparia sp.* | 3 | 1 | 1 |
| | Elachistidae | *Agonopterix sp.* | 3 | 1 | 1 |
| | | *Semioscopis sp.* | 2 | 0 | 1 |
| | Erebidae | *Laspeyria flexula* | 1a | 1 | 0 |
| | Geometridae | *Hydriomena impluviata* | 1a | 0 | 1 |
| | | *Idaea biselata* | 1b | 0 | 0 |
| | Momphidae | *Mompha epilobiella* | 1a | 0 | 1 |
| | Noctuidae | *Apamea monoglypha* | 1a | 1 | 0 |
| | | *Elaphria sp.* | 2 | 1 | 0 |
| | | *Hoplondrina blanda* | 1a | 0 | 1 |
| | | *Leucania sp.* | 3 | 1 | 0 |
| | | *Noctua sp.* | 3 | 1 | 1 |
| | | *Nonagria typhae* | 1a | 1 | 0 |
| | Pterophoridae | *Geina sp.* | 2 | 1 | 1 |
| | Tortricidae | *Acleris forsskaleana* | 1a | 1 | 0 |
| | | *Epinotia demarniana* | 1a | 1 | 1 |
| | | *Epinotia sp.* | 3 | 0 | 1 |
| Ephemeroptera | Baetidae | *Baetis fuscatus* | 1a | 1 | 0 |
| | Caenidae | *Caenis horaris* | 1b | 1 | 1 |
| | | *Caenis sp.* | 3 | 1 | 1 |
| | Ephemerellidae | *unknown* | 3 | 1 | 1 |
| | Heptageniidae | *Eurylophella sp.* | 2 | 1 | 0 |
| | | *Heptagenia dalecarlica* | 2 | 1 | 1 |
| Trichoptera | Goeridae | *Goera pilosa* | 1a | 0 | 0 |
| | Leptoceridae | *Athripsodes albifrons* | 1a | 0 | 1 |
| | | *Athripsodes cinereus* | 3 | 1 | 1 |
| | Limnephilidae | *Ceraclea sp.* | 1b | 1 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| | | *Limnephilus stigma* | 1b | 0 | 1 |
| | Molannidae | *Molanna albicans* | 1a | 1 | 1 |
| | | *Molanna angustata* | 1b | 0 | 1 |
| | Phryganeidae | *Agrypnia varia* | 1a | 1 | 1 |
| Neuroptera | Chrysopidae | *Nineta sp.* | 3 | 0 | 1 |
| | Hemerobiidae | *Hemerobius pini* | 1a | 0 | 1 |
| | | *Hemerobius sp.* | 3 | 0 | 1 |
| Hemiptera | Corixidae | *Callicorixa praeusta* | 1a | 0 | 1 |
| | | *Paracorixa concinna* | 1a | 0 | 1 |
| | | *Sigara falleni* | 1a | 1 | 0 |
| | | *Sigara striata* | 1a | 1 | 0 |
| Coleoptera | Carabidae | *unknown* | 3 | 0 | 1 |
| | Scarabaeidae | *Copris sp.* | 2 | 0 | 1 |
| Plecoptera | Perlodidae | *Clioperla sp.* | 1a | 1 | 1 |

**Table 34 Bray-Curtis Index and Hamming Distance values calculated on the molecular presence-absence diet data of *Myotis dasycneme* (Mdas) and *M. daubentonii* (Mdau) and the associated sexes (F = female; M = male).**

| | Hamming Distance | | | | | |
|---|---|---|---|---|---|---|
| Bray-Curtis Index | | Mdas_F | Mdas_M | Mdau_F | Mdau_M | Mdas_total |
| | Mdas_F | | 65 | 118 | 61 | |
| | Mdas_M | 0.80 | | 121 | 46 | |
| | Mdau_F | 0.78 | 0.85 | | 115 | |
| | Mdau_M | 0.81 | 0.82 | 0.85 | | |
| | Mdau_total | | | | | 117 / 0.703 |

**Table 45 Simpsons diveristy and Species richness (=number of prey) calculated from the morphological dietary data.**

| | *Myotis daubentonii* | | *Myotis dasycneme* | |
|---|---|---|---|---|
| | female | male | female | male |
| Simpson's Index | 0.81 | 0.79 | 0.77 | 0.69 |
| | 0.82 | | 0.75 | |
| Species richness | 12 | 14 | 10 | 90 |
| | 16 | | 12 | |
| Pianka's Index | 0.97 | | | |

**Table 56: Prey occurrence in the morphologically analysed diet of *M. dasycneme* and *M. daubentonii*. We tested data with generalized liner model (GLM) and Tukey post-hoc test. Bold p values indicate significant differences and values in italics almost significant cases (p < 0.1).**

| Prey occurrence | | | | |
|---|---|---|---|---|
| Prey | *M. dasycneme* (*n* = 84) | *M. daubentonii* (*n* = 122) | *z* | *p* |
| Diptera | 1.2% | 8.2% | 1.647 | *0.099* |
| Nematocera | 17.9% | 26.2% | 0.264 | 0.792 |
| Chironomidae | 95.2% | 82.0% | -2.628 | **0.008** |
| Chironomid Pupae | 17.9% | 11.5% | -1.709 | *0.088* |
| Tipulidae | 9.5% | 10.7% | 0.264 | 0.792 |
| Brachycera | 4.8% | 11.5% | 1.772 | *0.076* |
| Corixidae | 6.0% | 5.7% | 1.647 | 0.948 |
| Gerridae | 0.0% | 0.8% | 0.003 | 0.997 |
| Trichoptera | 46.4% | 50.8% | 0.619 | 0.536 |
| Lepidoptera | 14.3% | 12.3% | -0.416 | 0.678 |
| Ephemeroptera | 0.0% | 1.6% | 0.005 | 0.996 |
| Neuroptera | 1.2% | 4.1% | 1.146 | 0.252 |
| Coleoptera | 1.2% | 4.9% | 1.337 | 0.181 |
| Hymenoptera | 0.0% | 3.3% | 0.009 | 0.993 |
| Aphidoidea | 2.4% | 4.1% | 0.661 | 0.509 |
| Aranea | 0.0% | 0.8% | 0.003 | 0.997 |