Lakes as food sources for bats: evidence from stable isotopes and acoustic monitoring

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**Ithaka**

As you set out for Ithaka
hope the voyage is a long one,
full of adventure, full of discovery.
Laistrygonians and Cyclops,
angry Poseidon—don’t be afraid of them:
you’ll never find things like that on your way
as long as you keep your thoughts raised high,
as long as a rare excitement
stirs your spirit and your body.
Laistrygonians and Cyclops,
wild Poseidon—you won’t encounter them
unless you bring them along inside your soul,
unless your soul sets them up in front of you.

Hope the voyage is a long one.
May there be many a summer morning when,
with what pleasure, what joy,
you come into harbors seen for the first time;
may you stop at Phoenician trading stations
to buy fine things,
mother of pearl and coral, amber and ebony,
sensual perfume of every kind—
as many sensual perfumes as you can;
and may you visit many Egyptian cities
to gather stores of knowledge from their scholars.

Keep Ithaka always in your mind.
Arriving there is what you are destined for.
But do not hurry the journey at all.
Better if it lasts for years,
so you are old by the time you reach the island,
wealthy with all you have gained on the way,
not expecting Ithaka to make you rich.

Ithaka gave you the marvelous journey.
Without her you would not have set out.
She has nothing left to give you now.

And if you find her poor, Ithaka won’t have fooled you.
Wise as you will have become, so full of experience,
you will have understood by then what these Ithakas mean.

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Konstantinos P. Kavafis, 1911
Translated by Edmund Keeley/Philip Sherrard
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SUMMARY

This thesis deals with aquatic–terrestrial interactions, specifically in the use of freshwater insect fluxes by bats. Emerging aquatic insects are an important source of energy for bats and other terrestrial consumers. Many bat species depend on aquatic bodies not only for drinking water, but also partly or entirely for food. The main question of this thesis is how important are lakes as food sources for bats.

With a literature review (Chapter I), I first present an overview of the current knowledge on bats’ use of aquatic habitats, emphasizing how anthropogenic impacts on water bodies affect bats. This review shows that the majority of the studies have been conducted in Europe and North America, and most indirectly describe the use of aquatic resources from bats. The most common method used is acoustic monitoring, sometimes combined with other methods such as radio-tracking. It does not appear that research is focused on threatened or endangered species. The effects of water pollution and eutrophication on bats are unclear, as different effects are reported for different species and areas. Thus, this topic needs further investigation. More studies are also needed for understudied areas such as Africa, South America and Asia, and also for areas with limited water resources.

For the research of the topic, we used only non-invasive methods, for the bats. The first approach was biochemical tracers, i.e. stable isotope and fatty acid analysis of bat faeces. Since stable isotope analysis has so far been used in bat ecology mostly on hair, blood, muscles and claws, we wanted to explore faeces as samples, as they do not require catching the animals. First, we tested the effectiveness of the stable isotope analysis method on faeces, with a diet-shift experiment on captive bats of two species (Chapter II). We shifted the bats’ mealworm diet from light to heavy isotope labels and after seven days we shifted it back to the light isotopic label. The stable carbon (δ¹³C), nitrogen (δ¹⁵N) and sulphur (δ³⁴S) values on the faeces reflected the signature of the last diet within three hours after the last meal. We also calculated the isotopic difference (Δ) between diet and faeces which was significant for nitrogen, but not for carbon and sulphur, and did not differ for diet or species. These isotopic difference values are necessary for reconstructing diet from wild individuals and when the diet is unknown. Our results, that faecal stable isotopes reflect the isotopic signature of the last consumed food, showed that stable isotope analysis in faeces is a suitable method for investigating questions concerning short-term shifts in diet or habitat of bats, and possibly other insectivorous small mammals.

The next step was to apply these biochemical tracers on bat faeces from wild bats (Chapter III). Our question —always linked to the main question of this thesis— was to investigate whether it is possible to use stable isotopes on faeces to identify aquatic or terrestrial origins of
the prey. In addition to stable isotopes, we used another chemical tracer, fatty acids. Both stable isotopes and fatty acids have different signatures between different habitats, such as between aquatic and terrestrial systems. We collected fresh faeces from the roosts of three bat species with known preferences, on the borders of Germany and Switzerland. The species *Myotis daubentonii* is known to feed almost exclusively on aquatic insects (mainly Chironomidae), *M. myotis* feeds on terrestrial arthropods (beetles) and *M. mystacinus* has been reported to feed on both aquatic and terrestrial insects. Thus, we expected that the stable isotope and fatty acid values of their faeces would reflect their feeding preferences. In line with our expectations, we found higher $\delta^{15}$N and omega-3 and lower $\delta^{13}$C and omega-6 in *M. daubentonii*’s faeces, as is characteristic for freshwater systems. The opposite was true for *M. myotis*, while *M. mystacinus*—as expected—had intermediate values, indicating that it indeed feeds on both aquatic and terrestrial food.

The second approach, for investigating the relationship between bats and aquatic insects and shedding more light on the effect of season on aquatic insect emergence and bat activity, was a field study at three lakes in South Germany during the three seasons when bats are active (*Chapter IV & Chapter V*). We used passive acoustic monitoring, during the whole night, which is an effective, non-invasive method for assessing bat activity. For the nights of recording, as well as the preceding days, we collected emerging insects using floating emergence traps, and caught aerial flying insects using a Malaise trap. In all lakes, Chironomidae constituted the highest number of emergent insects; seasonal patterns of emergence were unimodal or bimodal with peaks at different times (beginning of summer, end of summer, beginning of autumn). Insect emergence had a positive relation with the water temperature in all lakes, but not with any other water parameters. In general, we found weak correlations between bat activity and insect emergence in the two lakes (Constance and Siechenweiher) and no correlations in Mindelsee. Bat activity also showed seasonal fluctuations that did not always follow insect emergence, probably because other factors (e.g., season, habitat characteristics, or energy requirements) played an important role. Bats were active throughout night, and the pattern of their activity also differed among lakes and seasons.

In conclusion, the results of this thesis show that lakes and their shores are important habitats for bats, as they support a high number of bat species. Aquatic fluxes to terrestrial systems have a considerable seasonal variation. Bat activity is influenced by season, insect availability and probably other factors (e.g., habitat structure, bats energy requirements) that we did not examine here. We suggest acoustic monitoring of bat activity and biochemical methods, i.e. stable isotope and fatty acid analysis of faeces, may be used to answer questions related to short-term diet or habitat shifts. These methods are non-invasive and efficient in studying aquatic-terrestrial trophic interactions and the use of aquatic resources by bats.
The findings of this thesis have a value for studying ecological questions related to food web dynamics, interactions between different habitats and animals or animal behaviour related to diet and habitat (with stable isotopes and fatty acids on faeces revealing short-term changes). The present results and conclusions may also prove useful for conservation, not only in the local region and for the studied species, but also for other insectivorous mammals or other species that rely on aquatic resources.
ZUSAMMENFASSUNG


Zusammenfassung

Isotope aus Kotproben dieselbe Signatur zeigen wie die letzte Mahlzeit des Tieres und dass diese Methode geeignet ist um kurzfristige Wechsel im Ernährungsschema oder des Habitats einer Fledermaus nachzuweisen. Gleiches sollte auch für andere kleine insektenfressende Säugetiere möglich sein.

Der nächste Schritt bestand darin, diese biochemischen Tracer auf Kotproben von wilden Fledermäusen anzuwenden (Kapitel III). Unsere Frage, in Anlehnung an die Hauptfrage der Dissertation, war es zu untersuchen ob sich mit stabilen Isotopen der aquatische oder terrestrische Ursprung der Ernährung identifizieren lässt. Zusätzlich zu den stabilen Isotopen haben wir Fettsäuren als weiteren chemischen Tracer benutzt. Sowohl stabile Isotopen als auch Fettsäuren unterscheiden sich im Hinblick auf verschiedene Habitats, so auch zwischen aquatischen und terrestrischen Systemen. Frischer Kot wurde entlang der Deutsch-Schweizer Grenze von den Schlafplätzen dreier verschiedener Fledermausarten mit bekannten Ernährungsgewohnheiten gesammelt. Die Wasserfledermaus, Myotis daubentonii, frisst fast nur aquatische Insekten (meistens Chironomiden). Das Große Mausohr, M. myotis, hingegen ernährt sich hauptsächlich von terrestrischen Arthropoden. Die Kleine Bartfledermaus, M. mystacinus, nutzt sowohl aquatische als auch terrestrische Insekten als Nahrungsquelle. Daher erwarteten wir, dass die Werte von stabilen Isotopen und Fettsäuren aus dem Kot die Ernährungsvorlieben der Fledermäuse reflektieren. Gemäß unserer Erwartungen fanden wir einen höheren Anteil an $\delta^{15}$N und Omega-3-Fettsäuren und einen niedrigeren Anteil an $\delta^{13}$C und Omega-6-Fettsäuren im Kot von M. daubentonii, was charakteristisch für Süßwassersysteme ist. Das Gegenteil war der Fall für M. myotis, während M. mystacinus wie erwartet Werte aufwies, die auf aquatische und terrestrische Ernährung zurückzuführen sind.

Ein zweiter Ansatz zur Untersuchung der Beziehung zwischen Fledermäusen und aquatischen Insekten legte Augenmerk auf den Effekt der Jahreszeit auf die Emergenz aquatischer Insekten und auf die Fledermausaktivität. Dazu wurde eine Feldstudie während der drei Jahreszeiten zu denen Fledermäuse aktiv sind an drei Seen in Süddeutschland durchgeführt (Kapitel IV & Kapitel V). Akustisches Monitoring während der ganzen Nacht diente als effektive und nicht-invasive Methode, um Fledermausaktivität abzuschätzen. Während der nächtlichen Aufnahmen und auch während den vorhergehenden Tagen wurden die aus den Seen schlüpfenden Insekten mit schwimmenden Emergenzfällen gesammelt, in der Luft fliegende Insekten mit einer Malaise-Falle. In allen Seen stellten die Chironomiden (Zuckmücken) den größten Teil der aquatischen Insekten dar; die jahreszeitlichen Muster der Emergenz waren unimodal oder bimodal mit Höhepunkten zu unterschiedlichen Zeiten (Beginn des Sommers, Ende des Sommers, Anfang Herbst). Im Allgemeinen fanden wir schwache Korrelationen zwischen Fledermausaktivität und Insektenemergenz für zwei Seen (Bodensee und
Siechenweiher) und keine Korrelation für den Mindelsee. Die Fledermausaktivität war jedoch auch jahreszeitlichen Schwankungen unterworfen, die nicht auf den Schlupf der Insekten zurückzuführen war, weshalb vermutlich auch andere Faktoren, wie Jahreszeit, Habitat eigenschaften und der Energiebedarf der Fledermäuse eine wichtige Rolle für die Aktivität von Fledermäusen spielen. Die Fledermäuse waren die ganze Nacht über aktiv, wobei sich die Aktivitätsmuster zwischen Seen und Jahreszeiten unterschieden.


GENERAL INTRODUCTION

Aquatic-terrestrial subsidies

Resources, the most important components of food webs, such as organisms, nutrients and detritus, can move between habitats (Polis et al. 1997). Subsidies are the spatial or allochthonous resources that originate in a donor habitat and enter into the food web of a recipient habitat, possibly altering its consumer-resource dynamics (Polis et al. 1997). For example, emerging aquatic insects are one type of subsidy that can be used for food by a number of terrestrial consumers such as spiders (Kato et al. 2003; Sanzone et al. 2003; Akamatsu et al. 2004), lizards (Sabo & Power 2002a,b), birds (Nakano & Murakami 2001; Uesugi & Murakami 2007) and bats (Fukui et al. 2006) (Fig. 1). Additionally, terrestrial insects that fall into water bodies often subsidize fishes (Kawaguchi & Nakano 2001; Baxter et al. 2004; Davis et al. 2010) (Fig. 1).

![A generalized diagram showing reciprocal flows of invertebrate prey and plant material input (dark arrows) that have direct and indirect effects on stream and riparian food webs (from Baxter et al. 2005).](image)

**Effects of subsidies**

Subsidies can have significant effects, be they positive or negative, direct or indirect, on both recipient and donor habitats (Polis & Hurd 1995; Polis et al. 1997). These can vary according to the taxa and behaviour of the organisms involved and the characteristics of the donor habitat system (Power & Rainey 2000). Such effects can include the modification of local consumer abundance, and the alteration of their effects on local prey (Sabo & Power 2002a,b). Moreover, allochthonous inputs can increase food-chain length (Pimm & Kitching 1987), influence food-
web stability (Huxel & McCann 1998; Takimoto et al. 2002), and affect energy, carbon and nutrient flow in a recipient system (Polis et al. 1997). Subsidies can be especially important for systems or periods with low productivity and low food availability. For example, when other food resources are limited, aquatic insect subsidies may be critical for the survival and reproduction of terrestrial bird populations (Nakano & Murakami 2001; Uesugi & Murakami 2007). Subsidies may also exhibit top-down or bottom-up effects in nearby ecosystems and induce trophic cascades (Nakano et al. 1999).

Subsidies effects on recipient communities may be altered when ecosystem modifications occur. Landscape-driven factors, such as flooding, not only control the magnitude of resource subsidies (aquatic insects), but also influence the ability of consumers (riparian spiders) to respond to them by altering the physical nature of the ecosystem boundary (Greenwood & McIntosh 2008).

The importance and challenges of studying subsidies
The importance of studying reciprocal subsidies lies in their abundance in ecosystems and the magnitude of their effects. The challenge is in understanding the factors that control the strength of the linkages between adjacent systems, the spatial and temporal extent of the subsidies, and their subsequent effects (Gratton et al. 2008). Information about subsidies is significant in understanding the processes that control the dynamics of species’ populations, communities, food webs and ecosystems (Polis et al. 1997). Knowledge of existing subsidies is also important in cases of introduced or invader species which can alter food webs and have other unexpected effects (Baxter et al. 2004; Finlay & Vredenburg 2007). Species invasions can interrupt the resource flow between connected ecosystems and have effects that propagate across their boundaries (Baxter et al. 2004). For example, introduced non-native trout in alpine lakes have outcompeted an alpine-nesting bird for emerging aquatic insect subsidies (Painter et al. 2009).

Due to the importance of subsidies to the recipient systems and their inhabitants, it is possible that the loss or degradation of one habitat may have more detrimental effects on neighbouring communities than might be expected (Nakano & Murakami 2001). The effects of these habitat alterations can be better understood if the occurring reciprocal subsidies are well studied. To conserve ecosystems it is essential to know and understand their structure, their function and the trophic relations not only inside one system but also along with the neighbouring systems. Detailed information about species’ foraging habitats, their feeding habits and the extent to which they are subsidized by allochthonous resources can assist with species conservation efforts.
Aquatic ecosystems such as freshwater and estuaries are the principal dietary sources of highly unsaturated fatty acids (HUFA) for all animals (Gladyshev et al. 2009). Emergent aquatic insects constitute one important way of exporting aquatic biomass, including HUFA, to terrestrial ecosystems. This adds to the importance of understanding subsidies from aquatic to terrestrial ecosystems. With HUFA playing a key role in the health of all organisms, studies that estimate the specific fluxes and accumulation of HUFA from particular aquatic ecosystems to their surrounding terrestrial ecosystems are needed (Gladyshev et al. 2009) in order to quantify the flow and the distribution of HUFA to different organisms.

**The importance of studying bats’ use of aquatic resources**

Bats are animals with a high value to ecosystems. They consume great amounts of arthropods, such as mosquitoes and agricultural pests, they play an essential role in pollination and seed dispersion, and their guano can be a source of nutrients, especially important in unproductive areas (reviewed by Kunz et al. 2011).

Although difficult to study due to their nocturnal and cryptic habits, bats are interesting animals to use as models. The importance of studying aquatic subsidies in relation to bats is that many bats species that are known to extensively rely on or supplement their diet with aquatic resources are highly relevant to conservation. For example, all European bat species are strictly protected and listed in the Annex IV of the Council Directive 92/43/EEC 1992 on the Conservation of Natural Habitats of Wild Fauna and Flora (EC Habitats Directive 1992). Other bat species are listed in Annex II, which lists animal and plant species of community interest, the conservation of which requires the designation of special areas for conservation (Bat Conservation Trust 2007).

**Methods of studying aquatic-terrestrial subsidies**

A variety of methods can be applied to study the fluxes of material between adjacent systems and their importance: mesocosm or field experiments (Lennon 2004; Hoekman et al. 2012); labelling resources and tracing (Sanzone et al. 2003); using natural tracers such as chemical components (e.g., stable isotopes and fatty acids); trapping animals while they enter the recipient system; animal diet investigations to see whether and how much they feed on allochthonous resources (Nakano & Murakami 2001); and monitoring animal activity (Nakano & Murakani 2001; Russo & Jones 2003) to see where foraging takes place. Hereafter, the methods (stable isotopes, fatty acids and acoustic monitoring), and the study animals (bats and insects) that were used in this thesis are discussed further.
Biochemical methods / tracers

Stable isotopes
Stable isotope analysis has been used to investigate and describe food-web structures in a number of ecological studies (e.g., Peterson & Fry 1987; reviewed in Fry 2008; Vander Zanden & Rasmussen 2001). Stable nitrogen isotope ratios ($\delta^{15}$N) in particular give information on trophic levels, as consumers usually have higher $\delta^{15}$N than diet due to the biochemical processes that take place during digestion. Stable carbon isotope ratios ($\delta^{13}$C) differ between different types of plants and provide information about diets and habitats, whilst sulphur stable isotope ratios ($\delta^{34}$S) relate to salinity (Fry & Chumchal 2011). Stable isotopes from all of the elements mentioned above differ between unpolluted water and sewage (Spies et al. 1989).

Stable isotope methods can be used to distinguish between terrestrial and aquatic sources (Phillips & Gregg 2001) and have previously been used to study aquatic subsidies to terrestrial systems (Collier et al. 2002; Farina et al. 2003; Paetzold et al. 2005; Gratton et al. 2008). For example, stable isotopes have been used to determine the relative proportions of terrestrial and marine subsidies of carbon to invertebrates along a tidal gradient, and to determine the relative importance of terrestrial carbon in food web pathways (Romanuk & Levings 2010). Stable isotopes can also be used as tracers in order to quantify the flow of carbon and nitrogen from aquatic to terrestrial systems with emerging aquatic insects (Sanzone et al. 2003), as well as the proportion of aquatic insects in the diets of riparian terrestrial predators such as arthropods (e.g., Briers et al. 2005; Paetzold et al. 2005).

Fatty acids
Fatty acids are parts of lipids; they are not degraded during digestion but instead accumulate over time, and can thus reflect dietary information for different time scales. They can be synthesized or modified by organisms, but there are limitations in these processes that differ between phylogenetic groups and species (Iverson 2009). Fatty acids can be measured relatively easily, and are sensitive to changes (Iverson 2009). These characteristics are advantageous in their use as trophic tracers and to study food web dynamics.

Fatty acids can be unsaturated, monounsaturated, polyunsaturated or highly saturated. Polyunsaturated fatty acids (PUFA) are important for animals, because they cannot usually be synthesized by animals but can be inserted into their diet. Omega-3 ($\omega3$) and omega ($\omega6$) PUFA, which differ in the position of their double bond, are two groups of PUFA that are essential for animals and need to be taken with food. The concentrations of these PUFA differ between different habitats, making PUFA useful for tracking the movement of resources and investigating diet. For example, pronounced differences exist between marine and freshwater habitats, but also
between terrestrial and aquatic habitats in general. Terrestrial organisms usually have higher omega-6 PUFAs compared to aquatic organisms, while the opposite is true for omega-3 PUFAs (Koussoroplis et al. 2008; Fontaneto et al. 2011).

Emerging aquatic insects

Many aquatic insects have a benthic life as larvae, but after becoming pupae and then adults they emerge from the water and follow a terrestrial life. Thus, emerging insects are important vectors of aquatic biomass in terrestrial systems, although their impact usually decreases with their distance from the aquatic body (Bartrons et al. 2013). The spatial influence of lotic and lentic emerging insects may differ, and in the buffer zone of water bodies their biomass can be up to 100 times higher than the terrestrial insect production (Bartrons et al. 2013).

Patterns of insect emergence can vary temporally and spatially. Four basic emergence patterns have been identified: continuous (in permanent lakes and rivers near the equator), rhythmic (lunar emergence), sporadic, and seasonal (related to the temperature, in the temperate zone) (Corbet 1964). In temperate zones they often have two emergence peaks, in summer and in autumn (e.g., Smukalla & Meyer 1988) or only in spring (Uesugi & Murakami 2007). Emergence can also vary from year to year, between different species and water bodies.

Common insect groups that have species with aquatic larval and terrestrial adult life are the orders of Ephemeroptera, Trichoptera and Diptera, and from the latter the Chironomidae, Ceratopogonidae, Chaoboridae and Simuliidae families in particular (e.g., Smukalla & Meyer 1988). Species of the Chironomidae family, called non-biting midges, (Fig. 2), often form the highest percentage of emerging insects from lakes (Smukalla & Meyer 1988; Ivković et al. 2013).

Bats

Bats, their life, and factors affecting their activity

Bats are nocturnal mammals with high diversity in size, from about 1.7 gr (Craseonycteris thonglongyai, Kitti hog-nosed bat or bumblebee bat; Burns 2013) to 1.6 kg (Pteropus giganteus, Indian flying fox; Silbernagel 2005), foraging techniques and feeding habits. Bats feed on fruits,
nectar, insects, frogs, other bats or small birds and fish, with only 2-3 species feeding on blood. All European bat species are insectivorous, with two exceptions – the *Myotis capaccinii* (long-fingered bat) that also occasionally eats small fish (Biscardi et al. 2007; Aizpurua et al. 2013) and the *Nyctalus lasiopterus* (giant noctule) that preys on migratory passerine birds (Ibáñez et al. 2001).

There are more than 1300 bat species in the world (Fenton & Simmons 2014) with 52 species having been recorded in Europe (UNEP/Eurobats 2014). They hibernate to pass the winter, and most species are sedentary, although migratory species also exist such as *Nyctalus noctula* (noctule) and *Pipistrellus nathusii* (Nathusius’ pipistrelle). Another characteristic of bats is the daily torpor, which is the short-term drop of the metabolism that bats use when food resources are not abundant.

The activity of bats is highly influenced by temperature and food availability. The foraging activity of bats also depends on their energy requirements, which vary according to the life stage. Pregnant and lactating bats have higher energy demands and so they forage longer if necessary (Encarnação et al. 2010). Other factors that influence bat activity are environmental and weather effects, such as rain and wind (Erickson & West 2002; Ciechanowski et al. 2007). The habitats where bats forage are related to their wing morphology and maneuverability, as well as to the specialized feeding techniques and echolocation call characteristics of each species (Aldridge & Rautenbach 1987; Marinello & Bernard 2014).

**Hourly nocturnal activity pattern of bats**

Bats usually emerge soon after sunset. Emergence time is related to bats’ feeding habits, foraging strategy, predation risk, energetic demands (e.g., Jones & Rydell 1994; Duvergé et al. 2000). Bats can be active all night or fly for some time before returning to their roosts. They can also make multiple foraging trips during the same night depending mostly on energy requirements and prey availability (Aldridge & Brigham 1991; Rintoul & Brigham 2014). Bat activity usually shows two peaks, one after sunset and one just before sunrise. Different species might show different patterns of nocturnal activity, or even the same species might change its pattern seasonally or spatially (O’Donnell 2000; Ciechanowski et al. 2009). Knowing the nocturnal pattern of bat activity is useful in understanding the relationships between species. Bats often develop different foraging strategies, and might forage in different times to avoid competition.

It is important to have an impression of the pattern of the activity, during the night, of the species for monitoring and research purposes. For example, in order to record bats for a limited time, the appropriate time of night with the highest activity or highest species number must be chosen, depending on the aim of the study.
General Introduction

Bat species in the study area

In Germany there are at least 24 recorded bat species, 21 of which have a confirmed presence in Baden-Württemberg (Eurobats Report 2014). In Konstanz, south Germany (the study area of this thesis) there are 14 reported species (Hinweise LUBW 2013). All four Pipistrellus species present in Europe can be found: Pipistrellus pygmaeus (pygmy pipistrelle), P. pipistrellus (common pipistrelle), P. nathusii and P. kuhlii (Kuhl’s pipistrelle). A number of Myotis species occur too: Myotis myotis (greater mouse-eared bat), M. daubentonii (Daubenton’s myotis), M. mystacinus (whiskered myotis), M. bechstenii (Bechstein’s bat) and M. nattereri (Natterer’s bat). Other species that are found are Nyctalus noctula, Plecotus auritus (brown big-eared bat), Pl. austriacus (gray big-eared bat), Eptesicus serotinus (serotine) and Vespertilio murinus (particoloured bat). The species N. leisleri (lesser noctule), Barbastella barbastellus (western barbastelle), Rhinolophus ferrumequinum (greater horseshoe bat), M. brandtii (Brandt’s myotis) and M. blythii (lesser mouse-eared myotis) have also been recorded in nearby regions in Switzerland (Fledermausschutz Thurgau 2014). The different species may also roost in different places, for example in human settlements, tree hollows or bat boxes.

All these species are insectivorous with varying degrees of specialization in their feeding habits and feeding strategies. Some species (e.g., M. daubentonii, P. kuhlii) prefer aquatic insects (e.g., Chironomidae, chironomids or non-biting midges) whilst other species (e.g., M. myotis, Pl. auritus) specialize in terrestrial arthropods, but most of them feed on both aquatic and terrestrial insects (e.g., P. pipistrellus, M. mystacinus). Each species might also be more or less generalist in their foraging habits (e.g., P. pipistrellus vs. P. pygmaeus, Russ & Montgomery 2002).

Bat echolocation – Acoustic monitoring

Bats produce ultrasonic sound calls. They use these high frequency (10-120 KHz) calls, also known as their sonar system, to orientate and to forage, by sending a call and locating the objects or the prey in their surrounding area using the echo that returns to them. Other types of call with lower frequencies (10-20 KHz) and different characteristics than the echolocation calls are the social calls, which are used for communication purposes. There are several types of social calls, including aggressive calls, mother-young interaction recognition calls, calls produced at the roost, and mating calls (Fenton 2003; Knörnschild et al. 2012).

Echolocation calls (Fig. 3) are usually species-specific and differ in their frequency characteristics, duration, structure, inter-pulse time, and volume. Species can be divided in groups according to their types of calls – there are species producing high frequency and short calls, while others produce low frequency and longer-duration calls. Calls can also vary even within species or individual according to the density of the habitat or the activity of the bat. A bat flying
in an open space usually produces lower frequency calls at longer intervals, while the opposite is true for a bat flying in a dense area where the need to locate objects is constant.

![Spectrogram](image)

**Fig. 3** Spectrograms of echolocation calls of *Pipistrellus pipistrellus* and *Myotis* bat species.

The ultrasound calls of bats can be used to monitor bat activity and species presence. Specialized passive and active recording systems have been developed (Fig. 4) that can record the ultrasound calls emitted from bats and make them audible to the human ear. Additionally, specialized software is being created that helps in identification of the bat calls or can even identify species or groups of species automatically. However, it is often still impossible to decide to which species to attribute a call, as some species have very similar calls (for example *Pipistrellus nathusii* and *P. kuhlii*) or the same individuals or species can produce different calls according to the environment and to their ‘personal’ differences. Bats also change the frequency of their echolocation calls when there are conspecifics flying in the same area, to avoid jamming (Ulanovsky et al. 2004; Bates et al. 2008). The quality of the recording is determinative for the identification of species, as other noises or echoes might interfere. In general, for the identification of species from bat calls it’s critical to take habitat characteristics into consideration. Using additional methods such as visual identification of bats that are trapped in mist nets or identified by night cameras can help to give more accurate results. Acoustic monitoring is also useful as a non-invasive method that doesn’t disturb bats, which is especially important for endangered or rare species. With recording only, however, the presence of some species might be missed or underestimated due to the fact that they fly too high or that their call is too low to be recorded.
General Introduction

Recording calls of bats does not give an estimate of the number of bats flying, but an estimate of the activity of bats and which species are present at the specific time and space. These activities can be performed by one bat flying all the time or by a number of bats passing by together. Therefore, activity is usually expressed according to the time or sequences of calls during a recording.

**Fig. 4** Bat recording device (batcorder, Ecoobs) (left) and a batcorder on a pole recording bat activity at lake Constance (right).

**Aim and objectives of this Ph.D. thesis**

The aim of this Ph.D. thesis is to investigate the aquatic subsidies in terrestrial systems using bats as model organisms. To do this, three different approaches were used: a literature review; biochemical methods (stable isotope and fatty acid analysis of bat faeces); and fieldwork with bat acoustic monitoring and the collection of emergent insects from lakes.

Firstly, the use of aquatic resources from bats is presented in the form of a literature review (Chapter I). The methods used, the study species and regions, and the factors that seem to affect the bat activity around aquatic systems are discussed. Aquatic bodies are important for bats because they not only provide food but also drinking water. A particular focus of the literature review was on the consequences to bats of human impacts on water bodies, such as eutrophication. Implications for conservation are also discussed and gaps in the current knowledge are identified.

Next, the aquatic subsidies in the diet of bats were explored. Non-invasive approaches were used for this study and so it was decided to use stable isotope and fatty acid analysis of faeces coupled with acoustic monitoring of bats. As stable isotope methods have not been used much in faeces of bats and other mammals, the efficiency of the method was tested first. A feeding
experiment with captive bats that were fed isotopically distinct labelled food was conducted (Chapter II). The stable carbon, nitrogen and sulphur isotopes in the faeces were measured before and after diet switches to determine when new isotope values would appear and to calculate the isotopic difference between diets and faeces. This is necessary to reconstruct diet from faeces of bats in the wild.

Then this method, stable isotopes in the faeces of wild bats was used, to trace aquatic versus terrestrial sources in their diet in combination with another one biochemical method, the analysis of fatty acids in faeces (Chapter III). Fresh faeces were collected from the roosts (Fig. 5, Fig. 6) of three bat species of the same genus living in the same area and with different feeding habits.

According to the literature, *Myotis myotis* is specialized on eating terrestrial crawling insects (beetles); *M. daubentonii* feeds predominantly on aquatic dipterans (Chironomidae), while *M. mystacinus* is known to feed on both (Arlettaz 1996; Vaughan 1997). It was hypothesized that stable isotope and fatty acid values in faeces will be different between the species *M. myotis* and *M. daubentonii*. *M. myotis* was expected to have a lower $\delta^{15}$N and higher omega-6 values, characteristic of the terrestrial systems in the area, with higher $\delta^{15}$N and higher omega-3 values expected for *M. daubentonii* as it is characteristic of the aquatic systems. For *M. mystacinus*, intermediate values between the other two species were expected due to its more general feeding characteristics.

Further indirect evidence of bats using aquatic systems for foraging or drinking water can be derived from acoustic monitoring near water bodies. By catching emergent insects, the number and biomass of aquatic insects available to bats and other terrestrial consumers can be assessed. For this project, the ultrasound calls of bats were recorded at the shores of three different lakes (Chapter IV). The aim was to monitor the bat activity at these lakes in relation to insect availability, and to search for common patterns in seasonal and hourly bat activity. The fieldwork was conducted during one ‘bat year’ (spring, summer, autumn) at the lakes Constance, Mindelsee and Siechenweiher, all located in South Germany. Insect emergence was monitored for the duration of this fieldwork by collecting emerging insects from these lakes every 5 days using floating traps (Fig. 7), as well as during the nights that the bat recordings were taking place. Aerial flying insects were also collected for a period of 3 months with a Malaise trap (Fig. 8). The insect abundance and biomass were correlated with the bat activity and the seasonal and hourly nocturnal pattern of bat activity is discussed.

More details, on the seasonal aquatic input from the three different lakes to the adjacent terrestrial systems, are provided in Chapter V. The relationship between the insect emergence rates and the water parameters are investigated and length-weight relationships are given for Chironomidae, the most abundant taxon.
At the end, a general discussion of all previous chapters, conclusions and future directions are presented.

**Fig. 5** Faeces of *Myotis myotis* on the floor of a church attic in Ermatingen, Switzerland.

**Fig. 6** The roost of *Myotis myotis* in the attic of a church in Ermatingen, Switzerland.
Fig. 7 Traps for emerging aquatic insects in Lake Mindelsee, Germany.

Fig. 8 The Malaise trap at the shore of Lake Mindelsee, Germany.
CHAPTER I

Bats’ use of aquatic habitats: a review emphasizing how anthropogenic impacts on water bodies affect bats

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Manuscript in review in Mammal Review

ABSTRACT

Many bats use aquatic habitats to feed and standing water to drink. Given that interactions between aquatic and terrestrial systems are important for understanding food web dynamics and for conserving species and ecosystems, this review examines the data available on bats’ use of aquatic habitats. One of the principal objectives was to assess how eutrophication and other anthropogenic impacts on water bodies affect bats. Most studies have been conducted in Europe and North America. They show, directly or indirectly, how bats use aquatic resources. Acoustic monitoring is the most common technique employed, although some studies have used radio-telemetry or other methods. *Myotis daubentonii* is the most commonly studied species. Research on this topic does not tend to focus more on threatened species (i.e., those included on the IUCN red list). I conclude that the effects of water pollution and eutrophication on bats remain unclear because different effects are reported for different species and different areas. Furthermore, more studies are needed from Africa, South America, and Asia, regions for which few data are available, as well as from arid regions where standing water is a limited resource.
INTRODUCTION

Organisms can move between ecological systems. For instance, aquatic insects can enter terrestrial food webs and be eaten by terrestrial consumers. It is important to characterize the extent of these trophic interactions between aquatic and terrestrial systems if we want to better understand food web dynamics. Such knowledge could be useful in assessing the effects of eutrophication or climate change, tracking pollutants, and determining the impacts of invasive species. Information on these interactions and on how resources move from one system to another (i.e., subsidies) is also important for creating conservation plans for species and ecosystems.

Bats, as well as other terrestrial consumers, depend on aquatic systems for drinking water (e.g., Adams & Hayes 2008); some species rely on them for food as well. Many studies provide indirect evidence of the relationship that exists between bats and aquatic insects. However, to date, there has been no systematic review of the data available on this topic. Information about the relationship between bats and aquatic systems is relevant for conservation efforts, especially with regards to threatened species and/or areas where water quantity or quality is limited from a bat’s perspective. Knowing the extent to which bats depend on aquatic resources may also help predict the effects of climate change or eutrophication, which are threats to freshwater systems. Also, contaminants or pollutants from aquatic systems can be transferred to terrestrial systems via bats’ consumption of insects.

The aim of the present review was to evaluate the importance of aquatic resources for bats and, in particular, to identify the effects that eutrophication, water pollution, and other anthropogenic impacts on water bodies have on bats.

The specific objectives were to: i) identify the characteristics of water bodies that make them more attractive to bats and search for any general patterns; ii) characterize any trends with regards to study methodologies (e.g., sampling location, species, or techniques used); iii) assess whether there are seasonal differences in the use of water bodies; iv) search for sex-specific or reproduction-related differences in the use of aquatic resources by bats; v) identify the effects of eutrophication or other anthropogenic impacts on water bodies on bats; vi) identify gaps in our current knowledge of bats’ use of aquatic resources; and vii) suggest topics that should be the focus of future research. The peer-reviewed literature was searched for direct and indirect evidence that bats use lakes, rivers, streams, coastal areas, wetlands, and ponds for feeding; it was not possible to exclude cases in which bats may be using water bodies only for drinking.
METHODS

I used the electronic database “Web of Science” to compile peer-reviewed studies on the topic of bats and their use of aquatic systems. I searched for papers in English mentioning the words bat(s) or Chiroptera and one of the following other words: aquatic insect(s), fish(es), lake(s), river(s), stream(s), canal(s), coastal, pond(s), wetland(s), marine, sea, riverine, aquatic, or foraging and water. Occasionally I subsequently included papers that were cited in the papers found via my search. I kept papers that referred to bats’ use of aquatic systems and excluded those in which this relationship went in the opposite direction (i.e., bats’ impacts on water bodies). I also excluded studies in which visual examinations of faeces were the only approach used to characterize the contribution made by aquatic insects to bats’ diets; there are many such studies, and they have been included in other reviews on bat feeding habits (e.g., Vaughan 1997; Safi & Kerth 2004). I included papers published up through May 2014.

The papers I found were scanned, and the information they contained—such as the species studied, the sampling location, the type of habitat studied, the duration of the study, the season(s) in which the study took place, the study systems examined, and the methods used—was extracted, grouped, and summarized in tables.

RESULTS AND DISCUSSION

A total of 150 studies were found. They concluded directly (59%) or indirectly (41%) that the bats, or some of the bats, being studied depended on aquatic prey or water resources. In many cases, it was impossible to determine whether the bats were using the water systems to feed or just to drink. Studies in which bats were reported to forage over or close to water (i.e., in riparian habitats) were also included as they implied bats were using water resources.

Because the studies varied greatly in their aims, focuses, methods, sample sizes, study species, study seasons, and study habitats, it was difficult to discern any general patterns, which was one of the initial objectives of the review. Hereafter, the findings are organized by topic, and the most relevant examples (and those that best fit with the review’s other objectives) are mentioned or discussed further. All the papers found are listed in Table 1.

Study locations and the ecosystems studied

The study locations were not evenly distributed geographically. My findings concur with those from the meta-analysis conducted by Bartels et al. (2012), which looked at reciprocal subsidies between freshwater and terrestrial ecosystems: it is obvious that more studies have
looked at systems in the Northern Hemisphere, especially in Europe and North America (47% and 31% of all studies, respectively). Only 7% of the studies were conducted in Asia, 5% in South America and Australia, and just 4% in Africa. These percentages are extremely small, especially considering the high species diversity found in these regions and the surface areas they cover. One reason why there are fewer studies for South America could also be that many South American bat species are frugivorous or nectarivorous (Kalko & Handley 2001; Sampaio et al. 2003) and thus do not use water bodies for feeding. The trend observed here—more publications coming from specific regions and/or countries—is also reflective of the fact that the publication rate is higher in general in the world’s wealthiest countries. May (1997) broached the topic of the scientific wealth of nations (in science, medicine, and engineering) and reported that the seven top-publishing countries (between 1981–1994) were also the world’s seven largest economies. Half of all the studies were conducted in just five countries: the USA (22%), the UK (12%), Germany (10%), Spain (7%), and Canada (7%). Although English is, to a great extent, the language of science, it might not be a coincidence that English-speaking countries published the greatest number of studies because this review is based on papers found using Web of Science and papers in languages other than English would therefore not have been found by the search.

Most studies (68%) looked at lotic systems; these systems include rivers (35%), streams (23%), and canals (10%). A considerable number of studies have looked at lentic systems, such as ponds (30%) or lakes (17%), or have examined riparian habitats (21%). It was less common for bat foraging activity to be studied near wetlands (7%), coastal areas (5%), or at sea (3%). Studies often focused on multiple habitats, either for comparative purposes or because bats were followed using radio-tracking and found to forage in several habitats.

There is limited evidence that bats use marine resources. However, when bats migrate, they may feed on marine insects or crustaceans (Hatch et al. 2013); indeed, the contribution of such prey to bat diets might be underestimated (Ahlén et al. 2009). Small bats, which have high-frequency echolocation calls, were found to feed on mosquitoes and emerge in large numbers, especially in coastal areas and marshlands (Gonsalves et al. 2013a,b). One exceptional case is the common vampire bat, Desmodus rotundus, which feeds on the blood of terrestrial mammals. Vampire bats feed on the blood of sea lions on some desert islands off the Peruvian coast; this resource represents an important marine subsidy that contributes to the survival of this terrestrial consumer (Catenazzi & Donnelly 2008). Riparian areas are important habitats for bats (e.g., Menzel et al. 2005a). Williams et al. (2006) found that half of the bat activity in a large study area occurred in riparian woodland, even though that specific habitat type accounted for less than 1% of all the riparian habitat present.
Several studies have examined bat activity over artificial wetlands or dams. Stahlschmidt et al. (2012) found that activity levels were higher over artificial ponds than over nearby vineyards, even though the ponds covered only a small percentage of the available area (<0.1%). Artificial wetlands and their adjacent riparian habitats also seem to be important foraging grounds for bats in agricultural landscapes in South Africa (Sirami et al. 2013).

Studies conducted in desert regions, which have limited water and vegetation, both temporally and spatially, have yielded interesting results. Williams & Dickman (2004) studied bat habitat use in a desert in Australia and found that temporary and permanent water bodies were the preferred habitats of almost all species. Furthermore, the most common species in a desert area in Israel were the non-desert species Pipistrellus kuhlii and Tadarida teniotis. Their distribution has expanded as human settlements have spread, and they make more visits to water resources to drink than do desert species (Razgour et al. 2010).

### Species studied and their feeding habits

Almost 1/3 (57) of the studies investigated a single species; the rest looked at two or more species or often the whole bat community found in a given area. The most frequently studied species (13% of all studies) was Myotis daubentonii, a common species in Europe. Myotis lucifugus, M. capaccinii, P. pygmaeus, and P. nathusii have also often been studied. Most of the species studied (>70%) are classified as species of least concern, according to the IUCN red list of threatened species; two species are near threatened, four are vulnerable, and one is endangered. For eight of the species, there is not enough information to determine their status. Also according to the IUCN, 9 species have populations in decline, while the rest have either stable (20 species), increasing (2 species), or unknown (12) population sizes. It does not appear that research on bats’ use of aquatic habitats is focused on threatened or endangered species.

Some of the species studied, such as M. capaccinii (e.g., Almenar et al. 2009) or M. daubentonii (e.g., Flavin et al. 2001) are more specialized or consume greater percentages of aquatic foods. Others, such as P. pipistrellus (e.g., Russ & Montgomery 2002; Lisón & Calvo 2013) and Eptesicus fuscus (Kalcounis-Ruepell et al. 2007), are opportunistic in their consumption of aquatic foods and/or demonstrate more generalist foraging habits.

Bats’ main aquatic prey are insects. Diptera (especially the family Chironomidae) and Trichoptera are among the insect orders preferred by bats. This information is usually obtained by visually identifying insect remains found in bat faeces (e.g., Flavin et al. 2001; Safi & Kerth 2004). Few studies use other methods or provide direct evidence that bats feed on aquatic insects. For example, the feeding habits of M. daubentonii were observed using spotlighting, and the species was seen catching insects over water surfaces (Dietz et al. 2006). Furthermore, Fukui et
al. (2006) experimentally reduced the number of insects emerging from a stream and thus demonstrated how important aquatic insects are for bats.

Aquatic insects are often a patchily distributed and ephemeral prey. Thus, it has been suggested that bats’ use of this food resource could have driven the evolution of their social foraging behaviour. Bats that feed on ephemeral prey might have had an advantage when foraging in a group since it is easier to detect swarming insects by listening to the calls of conspecifics than by individually detecting insects via echolocation. For example, the insectivorous species *Molossus molossus* forages in groups more often than expected by chance (Dechmann et al. 2010). Moreover, insect swarms are large enough to feed a group of jointly foraging bats; it is perhaps for this reason that *P. pipistrellus* socially forages on large insect swarms (Racey & Swift 1985). Males of bat species that feed on ephemeral prey (which mainly belong to the aquatic orders Ephemeroptera and Trichoptera) and that are morphologically adapted to fly in open areas are more likely to forage in groups (Safi & Kerth 2007).

Fish are another aquatic resource exploited by bats. For instance, *M. capaccinii* (Aihartza et al. 2008), *M. ricketti* (Ma et al. 2003), *Noctilio leporinus* (Bordignon 2006), and *N. albiventris* (Kalko et al. 1998) are piscivorous species. *Myotis vivesi* feeds almost exclusively on marine fishes and crustaceans and relatively rarely consumes terrestrial insects (Otalora-Ardila et al. 2013). Some bat species (e.g., *Megaderma zyra*) have also been reported to eat frogs (Marimuthu et al. 1995).

Methods used
The most commonly used method (57% of studies) was acoustic monitoring, whether passive or active; it was often combined with other methods, such as radio-tracking (21%) or mist-netting (32%). Visual observations, bat counting, video recording, and visual identification as well as molecular analyses of faeces and biochemical methods, such as stable isotope and fatty acid analyses, have also been used, but to a lesser extent.

Acoustic monitoring has the advantage of being non-invasive. Using passive recording systems, long-term data can be collected without the need for people to be present. If, additionally, software is used to automate call identification, the number of person-hours needed for analyses can be minimized. However, some species cannot be easily identified based on their calls, if at all. The use of calls can also be problematic for other reasons. For instance, if bat calls are not loud enough or a species tends to fly high, then it may be difficult or even impossible to obtain recordings of good quality (e.g., Jones & Rydell 1994; Jensen & Miller 1999). However, acoustic monitoring data by themselves cannot conclusively show whether bats are using aquatic systems for foraging or drinking, or whether they are simply commuting over them. However,
when feeding buzzes (Griffin 2001) are recorded, it is highly likely that bats are foraging. Forty-one percent of the studies using acoustic monitoring also used feeding buzzes to infer that bats were foraging.

Radio-tracking has certain advantages over acoustic monitoring. For example, researchers can analyse sex-specific and reproduction-related patterns of habitat use because bats must be caught to place the radiotrackers and can thus be identified as juveniles, males, or females. Also the relative use of different habitats by individual bats over the course of a given night can be assessed. However, it is hard to extensively characterize bat activity using radio-tracking because, typically, the number of bats being radiotracked is low and other bats without radiotrackers might be using the same habitats at the same time (Almenar et al. 2013). Mist-netting is most commonly used to catch bats to place tags or radiotrackers or to take faecal or tissue samples; it is less commonly used to determine which species are present in a given area. While mist-netting reveals information about the habitat use of individual bats, it cannot provide information about levels of foraging activity.

Although stable isotope analysis has been widely used in bat ecology to investigate migration or diet (e.g., Popa-Lisseanu et al. 2012; Cryan et al. 2012), only three studies have used this method to investigate bat use of aquatic systems. Bats feeding on aquatic insects have aquatic isotopic signatures in their faeces, as well as high levels of omega-3 fatty acids, which is common for organisms exploiting aquatic systems (Lam et al. 2013). Stable isotopes have also been used to discriminate between freshwater and marine contributions in the diet of *D. rotundus* (Catenazzi & Donnelly 2008) and *M. vivesi* (Otalora-Ardila et al. 2013).

Molecular techniques have also rarely been used, although the presence of aquatic insects in the diet of *M. daubentonii* (Vesterinen et al. 2013) was revealed via molecular analyses of the bat’s faeces. This method can even provide species-specific data about food items, if there is a DNA library available for the potential prey. Furthermore, such techniques can determine whether bats have been eating prey species that are known to tolerate pollution and can thus reveal the type and quality of the aquatic systems in which bats have been foraging (Clare et al. 2013).

Few studies have used experimental approaches (e.g., Fukui et al. 2006). Siemers et al. (2001) showed experimentally that *M. daubentonii* can catch fish. The potential for using experimental studies to examine aquatic-terrestrial interactions might be limited because of the challenges involved in working with bats and in manipulating the relatively large area over which bats forage.

Observations of bats obtained using light sources or image intensifiers (Van de Sijpe et al. 2004) and video recording (Razgour et al. 2010) have also provided direct evidence about bat feeding strategies. *Myotis daubentonii* was filmed catching its prey over a water surface (Todd &
The specific method used to study bat use of aquatic resources should depend on a study’s main research questions and aims, the study area, and the study species. For example, if it is important to use a non-invasive method and there is no need to identify the prey species being consumed, then stable isotope or fatty acid analyses of faeces should be preferred and are sufficient to determine whether or not aquatic food resources are being exploited. If the aim is to determine which specific aquatic systems in the study area are being used by bats (e.g., rivers vs. lakes) and the isotope signatures of the systems do not significantly differ, then other methods such as acoustic recording or radio-tracking might be more appropriate. If detailed information on the diet is needed, then molecular analyses of faeces can provide it. If researchers are specifically interested in bat behaviour, then video recording or experiments would be most helpful. However, the method used will also be determined by the availability of equipment, time, and personnel.

**Sex-specific and reproduction-related variation in the use of aquatic resources**

A few studies have investigated sex-specific differences in aquatic habitat use (e.g., Dietz et al. 2006). The roosts of male and female *M. daubentonii* were differentially distributed in terms of their distance to aquatic foraging areas (Encarnação et al. 2005). Compared to male roosts, female nursery roosts tended to be closer to water bodies and to occur at lower altitudes. In contrast, radiotracked male and female *P. nathusii* were found to have similar habitat preferences (Flaquer et al. 2009).

In *P. pipistrellus*, bats at different reproductive stages foraged in the same types of habitats; the only difference observed was that some bats foraged closer to their roosts when they were lactating (as compared to when they were pregnant) (Racey & Swift 1985). Although the authors focus on the use of water resources for drinking, the results found by Adams & Hayes (2008) are nonetheless interesting. They compared the number of visits made by lactating versus non-reproductive adult females to water resources in an arid region. They found that lactating bats made more visits. This finding shows that water resources are crucial for reproduction, especially in areas with limited water.

**Seasonal differences in the use of aquatic resources**

Few studies (14%) have assessed bats’ use of aquatic resources across seasons. Often fieldwork is performed for 2–3 months, during periods of higher activity or when bats are going through reproductive stages such as pregnancy, lactation, or post-lactation (e.g., Almenar et al. 2013; Clare et al. 2013).
The reason that bat activity has not been followed for longer periods of time might be that field work involving bats is demanding, especially when more labor-intensive methodologies are being used (e.g., radio-tracking). When such methods are used, studies usually last just a few nights or months. Alternatively, the aim is often to catch bats at their most active or focus on critical life stages. Furthermore, there is frequently a trade-off between collecting data over the long term at a few sites and collecting data over the short term at a greater number of sites or over two or three consecutive years. However, some studies (e.g., Zahn et al. 2008a) that spanned several months nonetheless chose to present mean activity for the entire period, masking seasonal differences.

Comparing bat activity over or near water bodies across seasons could be interesting because it might reveal that different species have different seasonal activity patterns. For instance, it seems likely that bats would be most dependent on aquatic insects during times when other food resources are limited, for example, in temperate regions in early spring (e.g., Fukui et al. 2006).

Aquatic insects, such as Chironomidae, can be multivoltine—hatching more than one clutch per year—and this life history trait can lead to large emergence events that take place several times a year, depending on the composition and abundance of the benthic communities involved. Bats, as well as other animals, can take advantage of these peaks (e.g., Fukui et al. 2006).

Seasonal differences in diet can be characterized in great detail using molecular techniques. *P. nathusii* eats different insects when it is migrating versus when it is occupying its summer roosts. Although its diet was still composed of aquatic insects, the species consumed more Chironomidae in the summer and more Tipulidae while migrating (Krüger et al. 2013). Furthermore, molecular analyses of faecal samples from different locations across Canada revealed that *M. lucifugus* consumes more (aquatic) Diptera in the early summer than later on in the year and that the diversity of species consumed differs seasonally (Clare et al. 2013).

**Characteristics of aquatic systems that affect their use by bats**

It has been found that several characteristics of aquatic habitats may affect their use by bats. The effects of the following features have been assessed: water system length, water system volume, number of months a water body held water (hydroperiod), distance to nearest permanent water source, water surface smoothness, vegetation cover, and site topography (e.g., Holloway & Barclay 2000; Biscardi et al. 2007; Francl 2008; Razgour et al. 2010). The relevant papers are listed in Table 2.

The same habitat feature can have different effects on different species at different locations. For instance, in an arid region, bat species richness and activity increased significantly
with pond size; species richness increased with pond length; bat activity increased with pond volume; and pond hydroperiod affected neither activity nor richness (Razgour et al. 2010) (Table 2). However, other patterns might occur in other systems. Moreover, because few studies have focused on the same species, it is difficult to make generalizations about species-specific patterns. One exception is *M. capaccinii*—it is one of the most commonly studied species and can be said to exhibit a general preference for linear water bodies (Biscardi et al. 2007) and rivers (Almenar et al. 2009) (Table 2).

Bats usually prefer certain habitats because of a combination of habitat characteristics; it is thus difficult to identify the contribution of any single characteristic. For instance, bat activity correlated poorly with features such as water depth, flow, and turbidity when these features were considered individually as opposed to when they were analysed in tandem (Johnson et al. 2010). Also, habitat structure (e.g., pool size and hydroperiod) as well as bat morphology can influence bat activity (Francl 2008).

The condition of the water surface (i.e., how turbulent and/or cluttered with vegetation it is) is a factor that influences the use of aquatic resources and is often studied. It can be generally concluded that bats usually prefer smooth water surfaces (e.g., *Myotis* spp.: Fenton et al. 1983; *M. lucifugus*: Mackey & Barclay 1989; *M. capaccinii*: Biscardi et al. 2007; *M. daubentonii*: Warren et al. 2000). However, while *E. fuscus*, a species that flies high, appears to dislike the noise of running water, it seems unaffected by cluttered water surfaces (Mackey & Barclay 1989).

The presence of riparian vegetation also seems to influence bat activity. Some riparian vegetation seems necessary; *M. capaccinii* avoided rivers that were cluttered or missing riparian vegetation, regardless of their width (Biscardi et al. 2007). In a prairie, bat activity was higher near and over parts of the river with trees than near and over those without trees, probably because trees provide shelter against the wind and rain and also food for insects and, as a result, harbor larger numbers of insects (Holloway & Barclay 2000). In contrast, in Mediterranean environments, bat foraging activity is higher near more accessible water bodies (Rainho 2007). In *M. daubentonii* and *P. pipistrellus* populations in the UK, bats did not show a preference for different relative numbers of trees at river sites (Rydell et al. 1994). However, the authors suggested that their findings differed from those of other studies possibly because they monitored bat activity during the dark phase of the night and not at twilight; it is at twilight that bats face the greatest predation risks and thus could benefit from more cover.

Few studies have dealt with the effects of altitude on bat use of water resources. Riparian habitats at lower elevations have higher levels of bat foraging activity than do riparian habitats at higher elevations (Grindal et al. 1999). Bat species richness seems to be higher near water bodies at higher elevations because water resources are less abundant at such altitudes (Johnson et al.
Another factor that is frequently left unexamined but that can affect the distribution and activity of bats is the proximity and availability of roosts (Holloway & Barclay 2000).

Of all the studies focused on bat foraging, only 21% relate bat activity to insect availability and, in most studies, terrestrial flying insects rather than emerging aquatic insects were collected. For instance, a study on *M. capaccinii* showed that the species demonstrated a preference for sites with high levels of insect availability (Almenar et al. 2009). However, it is often difficult to isolate the influence of insect availability since other factors play roles as well.

Few studies simultaneously took into account prey availability and habitat structure. However, Hagen & Sabo (2011), for example, investigated how aquatic and terrestrial insect availability and riverine habitat structure varied longitudinally and laterally (i.e., across river reaches) and how such variation simultaneously influenced bat activity. They found that the effect of habitat structure was greater than that of prey availability along rivers, while the opposite was true across rivers.

**How anthropogenic impacts on water bodies affect bats**

Various studies have examined the effects of anthropogenic impacts on water resources (e.g., eutrophication, organic pollution, and the construction of dams and sewage treatment facilities) on bats (Table 3). Most studies have focused on species that forage along and over rivers, and it is notable that almost all of them were conducted in the Northern Hemisphere and most in temperate regions (Table 3). In these areas, although water is not limited, eutrophication is a serious threat to many freshwater systems.

The effects of eutrophication and organic pollution have been found to differ among, and sometimes within, bat species (Table 3). *Eptesicus nilsonii* prefers eutrophic lakes, especially in July when general insect abundance is lower (DeJong 1994). Insect abundance and bat activity levels were found to be the same for a small eutrophic river and a large oligotrophic river (Racey et al. 1998). However, this result could be attributed to the fact that bat passes were only counted on a few nights (1 night per month for 3 months at 10 sampling sites per river) or because, as the authors noted, the level of eutrophication was not severe enough to cause sensitive caddis flies to disappear and tolerant chironomids to flourish. Vaughan et al. (1996) found that species reacted differently to eutrophication; although there was no clear pattern, *P. pipistrellus* and *P. pygmaeus*, but not *M. daubentonii* (and possibly other *Myotis* species), seemed to be negatively affected.

Kalcounis-Rüppell et al. (2007) studied the effects of wastewater treatment plants by investigating insect abundance, and bat foraging activity upstream and downstream from the plants. They found that, although total bat activity was the same both upstream and downstream, the treatment plants affected insect abundance and community structure as well as bat species.
distributions. Park & Cristinacce (2006) compared two different types of sewage treatment procedures and showed that one (filter beds) was better than the other (sludge) because it was associated with higher levels of bat activity (comparable to those at nearby riparian sites).

Biscardi et al. (2007) found that *M. capaccinii* preferred rivers of good quality in Italy (where quality was assessed using macroinvertebrates). However, elsewhere (in the Iberian Peninsula), the same species demonstrated a preference for lower water quality, although not all levels of water quality were available to the bat (Almenar et al. 2009). The same species can have more or less of a preference for aquatic habitats in different regions. For example, Lisón & Calvo (2013) compared their findings with those of others; they concluded that, although the presence of water bodies is a key determinant of the distributions of *P. pipistrellus* and *P. pygmaeus* in Mediterranean habitats, it does not influence their distribution patterns in other, more humid, regions of Europe.

Water quality (i.e., levels of organic pollution as assessed using physicochemical and biological criteria) had a negative or neutral effect on *M. dasycneme*, whose hunting activity was highest over sites with good and moderate water quality (Van de Sijpe et al. 2004). However, in the same study, different levels of water pollution did not appear to differentially influence the activity of *M. daubentonii*. The results for both species are qualitative in nature and do not take into account roost proximity. Another way water pollution can negatively affect bats is via mercury bioaccumulation. Higher concentrations of mercury were found in the fur, brains, and livers of bats collected near a mercury-contaminated river site as compared to at a reference site (Nam et al. 2012). Also, bats can be negatively affected by the pollutants present in their diets. Bat species known to prey on aquatic insects were found to have higher concentrations of heavy metals and metallothionein (a protein used to monitor levels of environmental heavy metal pollution) in their tissues (Pikula et al. 2010).

Climate change could affect how terrestrial consumers use aquatic resources since it will affect water temperatures. In a mesocosm experiment, warming and eutrophication resulted in higher numbers of emerging insects, while warming also caused insects to emerge earlier (Greig et al. 2012). The effects of warming on bats are discussed by Sherwin et al. (2013). Here, I only wish to address factors related to the disappearance of water resources, especially in arid regions. This phenomenon could be detrimental for bats, since they might have to travel longer distances to find water and would be exposed to the heat for longer periods and thus suffer from greater evaporative water loss, which could have negative effects on reproduction (Sherwin et al. 2013 and references therein). Climate change may make conditions riskier for bats found in areas where levels of water stress are higher and for bats that depend on prey whose availability varies temporally and spatially (e.g., aerial hawking bats) (Sherwin et al. 2013). It would seem that a
high percentage of European and West African bat species as a group are facing each of these two risks (32% and 81%, respectively) (Alcamo et al. 2007; Sherwin et al. 2013).

Not much data is available on how climate change and warming might impact bats that feed on aquatic insects. However, Adams & Hayes (2008), using field data and data compiled from the literature, created a model that shows how warming could impact lactating bats in arid regions. They predicted that, in an arid region like Colorado, an increase of only 1°C could reduce levels of stored water such that they could only support 64% of the area’s lactating females over their 21-day lactation period. Furthermore, according to the authors, this model may be conservative.

Bat activity was higher over heliponds (small ponds used by helicopters fighting forest fires) and ditches than in natural wetlands or pine forests, probably because the former are more open (with limited vegetation) and thus easier to access (Vindigni et al. 2009). This finding suggests that human-generated water resources might not always have negative effects on bats; indeed, under specific conditions and in certain areas, they might even be beneficial.

Fires, which can also result from human activities, can indirectly affect primary production in aquatic systems. A fire can sever or disrupt some of the links between aquatic and terrestrial systems. In spite of its relevance, this topic has been the focus of little research. It is important to characterize the types and levels of subsidies present in a given area if one wants to predict how they may be modified by fire. Malison & Baxter (2010) investigated the intermediate-term effects of fire on emerging aquatic insects and their predators (i.e., spiders and bats). They found that benthic and emerging aquatic insect biomass and bat activity were higher in riparian areas after high-intensity fires than after low-intensity fires; they were also higher than in unburned areas.

Another anthropogenic impact on aquatic systems that can indirectly affect terrestrial consumers such as bats is the construction of dams. The construction of a large dam in Portugal affected bats because it resulted in the loss of roost and foraging habitats and shifted bat activity from the area that became home to the dam to nearby riparian habitats (Rebelo & Rainho 2008). It is worth mentioning that artificial lighting can have a negative effect on adult aquatic insects because it affects their dispersal (Perkin et al. 2014). However, the indirect effects of such light pollution on bats—via aquatic insects—have yet to be studied.

Another interesting human impact on the availability of freshwater is the production of saline water, often containing cyanide and other toxic chemicals, by gold mining operations. A recent study (Griffiths et al. 2014) showed that, at goldfields in Australia, bats had higher levels of activity over cyanide-free fresh or brackish water bodies than over saline water bodies, whether or not the latter contained cyanide. Further research should examine these indirect effects of mining on bats.
Gaps in our knowledge and recommendations for future studies and conservation efforts

Clearly more studies are needed from Africa, Asia, and South America, regions for which available data are scarce. Also, there were many European countries for which I found few to no studies at all. For example, for countries with high bat diversity for Europe, such as Greece (in which occur 34 of the 52 bat species found in Europe; www.eurobats.org), studies on bats in general are limited in number and there are virtually no data available addressing how bats use water resources. Adams & Hayes (2008) mention that the use of water resources by bats in arid regions is underinvestigated and, based on the results of this review, I agree.

I support Seifert & Scheu’s (2012) statement that investigations of undisturbed ecosystems can help determine the significance of subsidies between aquatic and terrestrial systems. Thus, it is recommended that more studies be conducted in pristine ecosystems, to the degree to which that is possible. Such studies could establish a baseline that could be useful in the future when assessing the effects of anthropogenic factors, including global warming. More studies are necessary to fully evaluate how anthropogenic impacts directly affect aquatic ecosystems (e.g., the construction of dams) and thus indirectly affect bats. In particular, we still lack information on the relationship between eutrophication and bat activity and richness. We also need more studies that examine multiple stressors in aquatic systems and bats, in particular for desert regions. However, clearly characterizing these relationships might be difficult because there are many factors that affect bats, such as prey availability, the magnitude of eutrophication, and the nature of adjacent habitats. Large-scale experiments, although more demanding, might lead to a better understanding of the relationship between bats and their environment.

Most of the studies that measured bat activity near lakes or ponds focused on the shoreline or on riparian areas; sometimes, activity was also quantified over part of the lake or pond. However, studies that measure activity over open water are still lacking. It would be interesting to assess foraging activity over lakes and ponds as the results of such studies should clearly reveal the degree to which bats exploit aquatic insects. When bat activity is exclusively assessed along the shoreline, it is possible to overestimate bats’ utilization of terrestrial insects. Studies focused on streams have measured activity over the full width of the water course, probably because streams are narrower and thus easier to sample for technical reasons: bat detectors can provide fuller coverage and nets can be extended from one side of the stream to the other. We also need more studies that compare foraging activity over the water versus at the shore to truly understand bats’ use of aquatic insects; such studies should reveal the degree of bias introduced by exclusively sampling at the shoreline and how much feeding activity occurs over the open water.

Since most of the studies examining water quality were performed in lotic systems (i.e., rivers and streams) (Table 3), more studies should be performed in lentic systems (i.e., lakes and
ponds), especially since the latter face greater eutrophication risks. Studies on otherwise similar lakes that differ in their trophic conditions might help clarify the effects of eutrophication on bats. It could also be useful to further examine the impact of bats on mosquitoes. This is a topic of great interest as bats could serve as efficient biological controls of mosquito populations and thus limit disease transmission.

The relative importance of the quality versus the quantity of aquatic subsidies for bats remains to be investigated. A general literature review (i.e., not focused on bats) underscored the importance of both the quality and quantity of subsidies: it highlighted that subsidy use may depend not only on subsidy availability but also on subsidy quality (Marcarelli et al. 2011).

Another recommendation that emerges from this review is that more precise information on bat activity near or over aquatic systems could be gleaned by using a combination of methodologies and/or approaches. For instance, Razgour et al. (2010) used both acoustic monitoring and video recording to determine whether bats were drinking water or eating insects. Although they are often demanding and invasive for animals, more controlled and/or large-scale experiments might be useful because they could enhance our understanding of how bats are using aquatic resources. Further seasonal comparisons could yield different insights about the extent of bats’ use of aquatic resources. We need to conduct more studies that focus on endangered species, and possibly on species of varied conservation statuses, and that examine their responses to water resource modifications.

This review examined the degree to which bats use aquatic resources, the influence of aquatic resource characteristics on bat activity, and the impacts of anthropogenic modifications of aquatic systems on bat activity levels and distributions. The key words used for the literature search could have just as easily led to studies examining the opposite: how much bats influence aquatic systems. However, data on this topic are lacking; the few papers available deal with this question exclusively from the bat standpoint. For instance, bats could indirectly influence aquatic food webs via their predation on aquatic insects (Reiskind & Wund 2009); this research topic deserves further investigation.

Conclusions and conservation applications
This review shows that knowledge on bats’ use of aquatic resources is available but very fragmented. Most studies have been conducted in riverine systems and in only a few countries (i.e., the USA, the UK, and Germany) and focus on a limited number of species (e.g., *M. daubentonii*, *M. capaccinii*, and *M. lucifugus*). Although a variety of methods have been used, acoustic monitoring is the most common. Although some characteristics of aquatic systems, such as pond or river size, hydroperiod, insect availability, and habitat structure, have been found to be
important, it remains difficult to make general conclusions about species or habitats. Studies have examined the effects of eutrophication or other water quality issues on bats but have yielded conflicting results. More studies are needed to clarify how anthropogenic factors directly affect aquatic ecosystems and thus indirectly affect bats; their findings could guide conservation efforts aimed at at-risk species and ecosystems. Because climate change may shift species distributions and conservationists will thus face new challenges, it is essential to understand how bats are utilizing aquatic resources.

The relationship between water quality and bat activity is known for some bat species, such as *M. daubentonii*; consequently, they could be used as monitoring tools (Langton et al. 2010). Kalcounis-Rueppell et al. (2007) has suggested two species (*Perimyotis subflavus* and *E. fuscus*) that could serve as bioindicators of water quality because they are easy to track and their distributions are influenced by river wastewater levels. Molecular analyses of faeces also make it possible to detect whether bats have been eating insect species that are tolerant or sensitive to pollution and thus indirectly assess the water quality of the sites where bats are foraging (Clare et al. 2013).

As a result of this review, it is clear that aquatic resources are important for terrestrial consumers such as bats, including many bat species. They have diverse influences on different species and in different regions, and the ways in which anthropogenic impacts on aquatic systems affect bats are equally diverse.

**ACKNOWLEDGMENTS**

I am grateful to Karl-Otto Rothhaupt, Hans-Günther Bauer, Elizabeth Yohannes, and especially Mark Brigham for discussing and reviewing this manuscript. Thanks also to Yann Gager for some enlightening discussions and Jessica Pearce for her language editing services. I also wish to thank the University of Konstanz and the International Max Planck Research School for Organismal Biology, where I am a student, for giving me funding.
### Table 1. List of all studies found, the study species, habitat and country and reference.

<table>
<thead>
<tr>
<th>Species</th>
<th>Study habitats</th>
<th>Country and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corynorhinus townsendii</td>
<td>riparian</td>
<td>USA: Fellers &amp; Pierson 2002</td>
</tr>
<tr>
<td>Desmodus rotundus</td>
<td>islands</td>
<td>PERU: Catenazzi &amp; Donnelly 2008</td>
</tr>
<tr>
<td>Lasturus borealis</td>
<td>ponds, streams, reservoir, river, riparian</td>
<td>USA: Kurta &amp; Teramino 1992; Kalcounis-Ruepell et al. 2007; Walters et al. 2007; Brooks 2009; Lookingbill et al. 2010; Gilley &amp; Kennedy 2010</td>
</tr>
<tr>
<td>L. cinereus</td>
<td>pond, reservoir, river, riparian</td>
<td>CANADA: Holloway &amp; Barclay 2000; USA: Kurta &amp; Teramino 1992; Brooks 2009; Lookingbill et al. 2010</td>
</tr>
<tr>
<td>L. noctivagans</td>
<td>wetlands</td>
<td>USA: Lookingbill et al. 2010</td>
</tr>
<tr>
<td>L. bloisseevillii</td>
<td>riparian</td>
<td>USA: Calvert &amp; Neiswenter 2012</td>
</tr>
<tr>
<td>Macrophylum macrophyllum</td>
<td>lake</td>
<td>PANAMA: Meyer et al. 2005</td>
</tr>
<tr>
<td>Megaderma zyra</td>
<td>pond</td>
<td>INDIA: Marimuthu et al. 1995</td>
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<tr>
<td>Molossus molossus</td>
<td>stream</td>
<td>BRAZIL: Costa et al. 2011</td>
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<tr>
<td>Mormopterus norfolkensis</td>
<td></td>
<td>AUSTRALIA: McConville et al. 2014</td>
</tr>
<tr>
<td>Myotis auriculus</td>
<td>stream, lake</td>
<td>CANADA &amp; USA: Fenton &amp; Bell 1979</td>
</tr>
<tr>
<td>M. adversus</td>
<td></td>
<td>AUSTRALIA: Jones &amp; Rayner 1991</td>
</tr>
<tr>
<td>M. austroriparius</td>
<td>stream</td>
<td>USA: Gilley &amp; Kennedy 2010</td>
</tr>
<tr>
<td>M. ciliolabrum</td>
<td>river, riparian</td>
<td>CANADA: Holloway &amp; Barclay 2000</td>
</tr>
<tr>
<td>M. emarginatus</td>
<td>stream, riparian</td>
<td>GERMANY: Zahn et al. 2010</td>
</tr>
<tr>
<td>M. evotis</td>
<td>pond, river, riparian, lake</td>
<td>CANADA: Barclay 1991; Grindal et al. 1999; Holloway &amp; Barclay 2000</td>
</tr>
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Table 1. Continued from the previous page

<table>
<thead>
<tr>
<th>Species</th>
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<tbody>
<tr>
<td><em>M. frater</em></td>
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<td><em>M. lucifugus</em></td>
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<td><em>M. mystacinus/brandtii</em></td>
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<td><em>M. nattereri</em></td>
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<td><em>M. occultus</em></td>
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<td><em>M. ricketti</em></td>
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<tr>
<td><em>M. septentrionalis</em></td>
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<td><em>M. sodalis</em></td>
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<td><em>M. thysanodes</em></td>
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<td><em>M. vivesi</em></td>
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<td><em>M. volans</em></td>
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<tr>
<td><em>M. yumamensis</em></td>
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<tr>
<td>Neoromicia nana</td>
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<tr>
<td>Noctilio albiventris</td>
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<tr>
<td>N. labialis</td>
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<tr>
<td>N. leporinus</td>
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<tr>
<td>Nyctalus leisleri</td>
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<tr>
<td>Nyc. noctula</td>
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<tr>
<td>Nyctereis grandis</td>
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<tr>
<td>Nycticeius humeralis</td>
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<tr>
<td>Nyctinomops laticaudatus</td>
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<tr>
<td>Nyctinomops macrotis</td>
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<tr>
<td>Pipistrellus kuhlii</td>
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</tr>
<tr>
<td><em>P. nathusii</em></td>
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<table>
<thead>
<tr>
<th>Species</th>
<th>Description</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. subflavus</em></td>
<td>pond, reservoir</td>
<td>USA: Brooks 2009</td>
</tr>
<tr>
<td><em>Perimyotis subflavus</em></td>
<td>wetlands</td>
<td>USA: Lookingbill et al. 2010; Gilley &amp; Kennedy 2010</td>
</tr>
<tr>
<td><em>Plecotus auritus</em></td>
<td>sea, river, canal, pond, reservoir, stream, riparian</td>
<td>FINLAND: Wermundsen &amp; Siivonen 2008; JAPAN: Akasaka et al. 2010; UK: Swift &amp; Racey 1983; Rydell et al. 1996</td>
</tr>
<tr>
<td><em>Pteropus madanus</em></td>
<td>rice fields</td>
<td>INDIA: Senthilkumar et al. 2001</td>
</tr>
<tr>
<td><em>R. mehelyi</em></td>
<td>riparian</td>
<td>SPAIN: Salsamendi et al. 2012</td>
</tr>
<tr>
<td><em>S. leucogaster</em></td>
<td>stream, river, riparian</td>
<td>ZIMBABWE: Barclay 1985</td>
</tr>
<tr>
<td><em>V. murinus</em></td>
<td>lake</td>
<td>GERMANY: Haupt &amp; Schmidt 2007; SWITZERLAND: Jaberg &amp; Blay 2003</td>
</tr>
</tbody>
</table>
Table 2. Characteristics of aquatic systems that influence bat activity or species richness, system type, study species and region, reference cited, whether or not the characteristic had a significant effect (yes or no), data collected and method used, and notable results or other comments.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>System type (country)</th>
<th>Species</th>
<th>Effect</th>
<th>Data collected (method)</th>
<th>Reference</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>altitude</td>
<td>ponds, rivers, streams, rivers (USA)</td>
<td>6 species</td>
<td>yes</td>
<td>bat richness (recording)</td>
<td>Johnson et al. 2010</td>
<td>water bodies at higher elevations had greater bat richness</td>
</tr>
<tr>
<td></td>
<td>lakes (CANADA)</td>
<td>many</td>
<td>yes</td>
<td>bat activity: commuting, foraging (recording), bat captures (mist-netting)</td>
<td>Grindal et al. 1999</td>
<td>foraging activity high&lt;mid&lt;low, commuting activity higher: mid&lt;high&lt;low elevation. Captures higher at low and mid than higher elevation</td>
</tr>
<tr>
<td>water system size</td>
<td>livestock tanks (USA)</td>
<td>many</td>
<td>no</td>
<td>number of passes (camera)</td>
<td>Jackrel &amp; Matlack 2010</td>
<td>experiment</td>
</tr>
<tr>
<td></td>
<td>livestock tanks (USA)</td>
<td>many</td>
<td>yes</td>
<td>drinking (camera)</td>
<td>Jackrel &amp; Matlack 2010</td>
<td>(experiment) bats more often drank from the 3-m than the 1.2-m tank more activity at small and large pools than at medium pools</td>
</tr>
<tr>
<td></td>
<td>seasonal pools (USA)</td>
<td>many</td>
<td>yes</td>
<td>activity (recording, mist-netting)</td>
<td>Francl 2008</td>
<td></td>
</tr>
<tr>
<td>water depth</td>
<td>livestock tanks (USA)</td>
<td>many</td>
<td>no</td>
<td>number of passes (camera)</td>
<td>Jackrel &amp; Matlack 2010</td>
<td>fewer passes</td>
</tr>
<tr>
<td></td>
<td>livestock tanks (USA)</td>
<td>many</td>
<td>yes</td>
<td>drinking (camera)</td>
<td>Jackrel &amp; Matlack 2010</td>
<td>bats never drank from heavily obstructed tanks</td>
</tr>
<tr>
<td>abundant vegetation</td>
<td>livestock tanks (USA)</td>
<td>many</td>
<td>yes</td>
<td>number of passes (camera)</td>
<td>Jackrel &amp; Matlack 2010</td>
<td>fewer passes</td>
</tr>
<tr>
<td></td>
<td>livestock tanks (USA)</td>
<td>many</td>
<td>yes</td>
<td>drinking (camera)</td>
<td>Jackrel &amp; Matlack 2010</td>
<td>bats never drank from heavily obstructed tanks</td>
</tr>
<tr>
<td>water system length</td>
<td>ponds (ISRAEL)</td>
<td>many</td>
<td>yes</td>
<td>activity, species richness (recording, experiment)</td>
<td>Razgour et al. 2010</td>
<td>species richness increased logarithmically with pond size; activity was higher when ponds were longer</td>
</tr>
<tr>
<td>hydro-period</td>
<td>ponds (ISRAEL)</td>
<td>many</td>
<td>yes/no</td>
<td>activity, species richness (recording, experiment)</td>
<td>Razgour et al. 2010</td>
<td>bat activity affected by hydroperiod; when the temporary ponds dried up, activity and richness increased at other, more permanent ponds</td>
</tr>
<tr>
<td></td>
<td>seasonal pools (USA)</td>
<td>many</td>
<td>yes</td>
<td>activity (recording, mist-netting)</td>
<td>Francl 2008</td>
<td>the number of bat calls decreased as the amount of open water declined</td>
</tr>
<tr>
<td>pond volume</td>
<td>ponds (ISRAEL)</td>
<td>many</td>
<td>yes</td>
<td>activity (recording, experiment)</td>
<td>Razgour et al. 2010</td>
<td>total activity increased linearly with pond volume</td>
</tr>
</tbody>
</table>

Continued in the next page
<table>
<thead>
<tr>
<th>water system width</th>
<th>species</th>
<th>foraging activity (recording)</th>
<th>Van de Sijpe et al. 2004</th>
<th>the broader the waterway, the higher the activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>canals (BELGIUM)</td>
<td>M. dasycneme</td>
<td>yes</td>
<td>activity (radio-tracking)</td>
<td>Biscardi et al. 2007</td>
</tr>
<tr>
<td>river (ITALY)</td>
<td>M. capaccinii</td>
<td>yes</td>
<td>activity (radio-tracking)</td>
<td>activity was highest at river width of 5–10 m</td>
</tr>
<tr>
<td>rivers, canals, reservoirs, ponds (SPAIN)</td>
<td>M. capaccinii</td>
<td>yes</td>
<td>foraging activity</td>
<td>Almenar et al. 2009</td>
</tr>
<tr>
<td>tree density/ canopy cover</td>
<td>seasonal pools (USA) many</td>
<td>activity, species presence (recording, mist-netting)</td>
<td>Franci 2008</td>
<td>tree density (one measure of structural complexity) showed no trends: it was correlated with other factors; differences observed between years</td>
</tr>
<tr>
<td>density of riparian vegetation (trees along riverside)</td>
<td>rivers (UK)</td>
<td>M. daubentonii, P. pipistrellus, P. pygmaeus</td>
<td>no</td>
<td>number of bats (recording)</td>
</tr>
<tr>
<td>smoothness of water surface</td>
<td>rivers, canals, reservoirs, ponds (SPAIN)</td>
<td>M. capaccinii</td>
<td>foraging activity (radio-tracking)</td>
<td>Almenar et al. 2009</td>
</tr>
<tr>
<td>streams (CANADA)</td>
<td>M. lucifugus, E. fuscus</td>
<td>yes/ no</td>
<td>bat activity (recording, experimental conditions)</td>
<td>Mackey &amp; Barclay 1989</td>
</tr>
<tr>
<td>streams, ponds (CANADA)</td>
<td>M. lucifugus</td>
<td>yes</td>
<td>activity, presence (recording, mist-netting)</td>
<td>von Frenckell &amp; Barclay 1987</td>
</tr>
<tr>
<td>river (ITALY)</td>
<td>M. capaccinii</td>
<td>yes</td>
<td>activity (radio-tracking)</td>
<td>Biscardi et al. 2007</td>
</tr>
<tr>
<td>river (UK)</td>
<td>M. daubentonii, P. pipistrelli</td>
<td>yes</td>
<td>activity (recording)</td>
<td>Warren et al. 2000</td>
</tr>
<tr>
<td>roost proximity</td>
<td>rivers, springs, riparian zones (CANADA)</td>
<td>E. fuscus, Myotis spp.</td>
<td>yes</td>
<td>more activity closer to roosts (recording)</td>
</tr>
<tr>
<td>rivers, canals, reservoirs, ponds (SPAIN)</td>
<td>M. capaccinii</td>
<td>yes</td>
<td>foraging activity (radio-tracking)</td>
<td>Almenar et al. 2009</td>
</tr>
</tbody>
</table>

Continued in the next page
Table 2: Continued from the previous page

<table>
<thead>
<tr>
<th>presence of riparian vegetation</th>
<th>presence</th>
<th>activity</th>
<th>conditions in riparian zone*</th>
<th>habitat structure</th>
<th>channel confinement (riverine structure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rivers, canals, reservoirs, ponds (SPAIN)</td>
<td><em>M. capaccinii</em></td>
<td>yes</td>
<td>foraging activity (radio-tracking)</td>
<td>more activity over water bodies without riparian vegetation</td>
<td>higher activity and density in more confined areas</td>
</tr>
<tr>
<td>river (ITALY)</td>
<td><em>M. capaccinii</em></td>
<td>yes</td>
<td>activity (radio-tracking)</td>
<td>bats preferred well-developed vegetation and avoided the parts of the river that were either very cluttered or missing riparian vegetation</td>
<td></td>
</tr>
<tr>
<td>river (CANADA)</td>
<td><em>Myotis spp.</em>, <em>E. fuscus</em></td>
<td>yes</td>
<td>activity &amp; feeding buzzes (recording)</td>
<td>higher activity in areas with trees than without</td>
<td></td>
</tr>
<tr>
<td>ponds, rivers, streams, rivers (USA)</td>
<td>6 species</td>
<td>yes</td>
<td>activity (recording)</td>
<td>different correlations for different species</td>
<td>habitat structure and bat morphology affect activity: large-bodied, less maneuverable species more commonly forage in open habitats; sig. effects in tandem with other factors</td>
</tr>
<tr>
<td>river (CANADA)</td>
<td><em>Myotis spp.</em></td>
<td>yes</td>
<td>activity &amp; feeding buzzes (recording)</td>
<td>higher activity near steep coulees than in areas with gentle topography</td>
<td></td>
</tr>
<tr>
<td>river (CANADA)</td>
<td><em>L. cinereus</em></td>
<td>yes</td>
<td>activity (passes) (recording)</td>
<td>higher activity in areas with gentler slopes than near steep coulees</td>
<td></td>
</tr>
<tr>
<td>seasonal pools (USA)</td>
<td>many</td>
<td>yes</td>
<td>activity (recording, mist-netting)</td>
<td>Francl 2008</td>
<td></td>
</tr>
<tr>
<td>rivers (USA)</td>
<td>many</td>
<td>yes</td>
<td>activity, density (recording)</td>
<td>Hagen &amp; Sabo 2011</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Effects of anthropogenic impacts on aquatic systems on bat species: + = positive; - = negative; and 0 = neutral. Categorized by pollutant or impact type. Habitat, reference cited, country where the study was performed, and any relevant notes.

<table>
<thead>
<tr>
<th>Pollutant/impact</th>
<th>Species</th>
<th>Effects</th>
<th>Habitat (Country)</th>
<th>Reference cited</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>general anthropogenic impacts</td>
<td><em>P. nathusii</em></td>
<td>$\sqrt{+}$</td>
<td>wetland (Spain)</td>
<td>Flaquer et al. 2009</td>
<td>rice paddies vs. unaffected wetlands</td>
</tr>
<tr>
<td>degradation of riparian habitat</td>
<td><em>P. pipistrellus</em></td>
<td>$\sqrt{+}$</td>
<td>river (UK)</td>
<td>Scott et al. 2009</td>
<td>more feeding buzzes (but non significantly higher) at undisturbed sites</td>
</tr>
<tr>
<td></td>
<td><em>P. pygmaeus</em></td>
<td>$\sqrt{+}$</td>
<td>river (UK)</td>
<td>Scott et al. 2009</td>
<td>feeding buzzes and total activity</td>
</tr>
<tr>
<td>eutrophication</td>
<td><em>E. nilsonii</em></td>
<td>$\sqrt{+}$</td>
<td>lake; Sweden</td>
<td>deJong 1994</td>
<td>upstream and downstream from sewage treatment plants</td>
</tr>
<tr>
<td></td>
<td><em>E. serotinus</em></td>
<td>$\sqrt{+}$</td>
<td>river (UK)</td>
<td>Vaughan et al.1996</td>
<td>upstream and downstream from sewage treatment plants</td>
</tr>
<tr>
<td></td>
<td><em>M. daubentonii</em></td>
<td>$\sqrt{+}$</td>
<td>river (UK)</td>
<td>Racey et al. 1998</td>
<td>positive: increased bat activity; negative: bats bioaccumulated heavy metals from water upstream and downstream from sewage treatment plants</td>
</tr>
<tr>
<td></td>
<td><em>M. daubentonii</em></td>
<td>$\sqrt{+}$</td>
<td>river (UK)</td>
<td>Vaughan et al.1996</td>
<td>upstream and downstream from sewage treatment plants</td>
</tr>
<tr>
<td></td>
<td><em>M. daubentonii</em></td>
<td>$\sqrt{+}$</td>
<td>river (UK)</td>
<td>Vaughan et al.1996</td>
<td>upstream and downstream from sewage treatment plants</td>
</tr>
<tr>
<td></td>
<td><em>M. daubentonii</em></td>
<td>$\sqrt{+}$</td>
<td>river (UK)</td>
<td>Rydell et al. 1994</td>
<td>positive: increased bat activity; negative: bats bioaccumulated heavy metals from water upstream and downstream from sewage treatment plants</td>
</tr>
<tr>
<td></td>
<td><em>Neoromicia nana</em></td>
<td>$\sqrt{+}$</td>
<td>river (S. Africa)</td>
<td>Naidoo et al. 2013</td>
<td>upstream and downstream from sewage treatment plants</td>
</tr>
<tr>
<td></td>
<td><em>Nyctalus spp.</em></td>
<td>$\sqrt{+}$</td>
<td>river (UK)</td>
<td>Vaughan et al.1996</td>
<td>upstream and downstream from sewage treatment plants</td>
</tr>
<tr>
<td></td>
<td><em>P. kuhlii</em></td>
<td>$\sqrt{+}$</td>
<td>river (UK)</td>
<td>Vaughan et al.1996</td>
<td>upstream and downstream from sewage treatment plants</td>
</tr>
<tr>
<td></td>
<td><em>P. pipistrellus</em></td>
<td>$\sqrt{+}$</td>
<td>river (UK)</td>
<td>Racey et al. 1998</td>
<td>positive: increased bat activity; negative: bats bioaccumulated heavy metals from water upstream and downstream from sewage treatment plants</td>
</tr>
<tr>
<td></td>
<td><em>P. pygmaeus</em></td>
<td>$\sqrt{+}$</td>
<td>river (UK)</td>
<td>Vaughan et al. 1996</td>
<td>upstream and downstream from sewage treatment plants; higher activity upstream</td>
</tr>
<tr>
<td></td>
<td><em>P. pipistrellus</em></td>
<td>$\sqrt{+}$</td>
<td>river (UK)</td>
<td>Rydell et al. 1994</td>
<td>upstream and downstream from sewage treatment plants; higher activity upstream</td>
</tr>
</tbody>
</table>

Continued in the next page
### Table 3. Continued from the previous page

<table>
<thead>
<tr>
<th>Environmental Factor</th>
<th>Species</th>
<th>Habitat Type</th>
<th>Location</th>
<th>Activities/Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>organic pollution</strong></td>
<td><em>M. dasycneme</em></td>
<td>√</td>
<td>canal (Belgium)</td>
<td>Van de Sijpe et al. 2004</td>
</tr>
<tr>
<td></td>
<td><em>M. daubentonii</em></td>
<td>√</td>
<td>canal (Belgium)</td>
<td>Van de Sijpe et al. 2004</td>
</tr>
<tr>
<td><strong>sewage effluent</strong></td>
<td><em>M. daubentonii</em></td>
<td>√</td>
<td>river (Ireland)</td>
<td>Abbott et al. 2009</td>
</tr>
<tr>
<td></td>
<td><em>P. pipistrellus</em></td>
<td>√</td>
<td>river (Ireland)</td>
<td>Abbott et al. 2009</td>
</tr>
<tr>
<td></td>
<td><em>P. pygmaeus</em></td>
<td>√</td>
<td>river (Ireland)</td>
<td>Abbott et al. 2009</td>
</tr>
<tr>
<td><strong>urbanization</strong></td>
<td><em>E. fuscus</em></td>
<td>√</td>
<td>river; Canada</td>
<td>Coleman &amp; Barclay 2013</td>
</tr>
<tr>
<td></td>
<td><em>E. fuscus</em></td>
<td>√</td>
<td>river; USA</td>
<td>Kurta &amp; Teramino 1992</td>
</tr>
<tr>
<td></td>
<td><em>E. nilsonii</em></td>
<td>√</td>
<td>stream; Japan</td>
<td>Akasaka et al. 2010</td>
</tr>
<tr>
<td></td>
<td><em>Lasionycteris noctivagans</em></td>
<td>√</td>
<td>river; Canada</td>
<td>Coleman &amp; Barclay 2013</td>
</tr>
<tr>
<td></td>
<td><em>L. borealis</em></td>
<td>√</td>
<td>river; Canada</td>
<td>Coleman &amp; Barclay 2013</td>
</tr>
<tr>
<td></td>
<td><em>L. borealis</em></td>
<td>√</td>
<td>river; USA</td>
<td>Kurta &amp; Teramino 1992</td>
</tr>
<tr>
<td></td>
<td><em>L. cinereus</em></td>
<td>√</td>
<td>river; Canada</td>
<td>Coleman &amp; Barclay 2013</td>
</tr>
<tr>
<td></td>
<td><em>M. daubentonii</em></td>
<td>√</td>
<td>stream; Japan</td>
<td>Akasaka et al. 2010</td>
</tr>
<tr>
<td></td>
<td><em>M. lucifugus</em></td>
<td>√</td>
<td>river; USA</td>
<td>Kurta &amp; Teramino 1992</td>
</tr>
<tr>
<td></td>
<td><em>M. sodalis</em></td>
<td>√</td>
<td>river; USA</td>
<td>Kurta &amp; Teramino 1992</td>
</tr>
<tr>
<td></td>
<td><em>Myotis spp.</em></td>
<td>√</td>
<td>river; Canada</td>
<td>Coleman &amp; Barclay 2013</td>
</tr>
<tr>
<td><strong>wastewater</strong></td>
<td><em>Nycticeius humeralis</em></td>
<td>√</td>
<td>river; USA</td>
<td>Kalcounis-Rueppell et al. 2007</td>
</tr>
<tr>
<td></td>
<td><em>P. subflavus</em></td>
<td>√</td>
<td>river; USA</td>
<td>Kalcounis-Rueppell et al. 2007</td>
</tr>
<tr>
<td></td>
<td><em>E. fuscus</em></td>
<td>√</td>
<td>river; USA</td>
<td>Kalcounis-Rueppell et al. 2007</td>
</tr>
<tr>
<td><strong>poor water quality (biological)</strong></td>
<td><em>M. daubentonii</em></td>
<td>√</td>
<td>river, stream (UK)</td>
<td>Langton et al. 2010</td>
</tr>
<tr>
<td><strong>water quality</strong></td>
<td><em>M. capaccinii</em></td>
<td>√</td>
<td>river; Italy</td>
<td>Biscardi et al. 2007</td>
</tr>
</tbody>
</table>
CHAPTER II

Advantages of using faecal samples for stable isotope analysis in bats: evidence using a triple isotopic experiment

Ioanna Salvarina, Elizabeth Yohannes, Björn M. Siemers, Klemen Koselj

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ABSTRACT

Stable isotope analysis in ecological studies is usually conducted on biomaterials, e.g., muscle and blood that require catching the animals. Faeces are rarely used for stable isotope analysis, despite the possibility of non-invasive sampling and short-term responsiveness to dietary changes. This promising method is neglected due to a lack of calibration experiments and unknown diet-faeces isotopic difference ($\Delta_{\text{diet-faeces}}$). To fill this gap, we simulated trophic changes occurring in nature when animals switch feeding habitats, e.g., by moving from freshwater to terrestrial systems, from cultivated areas to forests or changing distance from marine environments. In a controlled experiment, the diet of two bat species, ($Myotis$ $myotis$, $Rhinolophus$ $ferrumequinum$), was altered to an isotopically distinct one. We measured stable nitrogen, carbon and the rarely used sulphur isotope in faeces, and calculated $\Delta_{\text{diet-faeces}}$ values. The faeces acquired the new dietary signature within 2-3 hours from food ingestion, thus they are suited for detecting recent and rapid dietary changes. The $\Delta_{\text{diet-faeces}}$ ($A$) did not differ between species or diet (overall means ± sds): $A^{15}N$: 1.47 ± 1.51‰, $A^{13}C$: -0.11 ± 0.80‰, $A^{34}S$: 0.74 ± 1.10‰. Only $A^{15}N$ for $M. myotis$ was significantly different from zero and only $A^{13}C$ differed among the days of the experiment. Faecal stable isotopes can be now further applied in mammalian ecology. This includes a range of applications, such as studying changes in trophic level, resource or habitat use, on a short time-scale. Such information is gaining importance for monitoring rapidly changing ecosystems under anthropogenic influence.
INTRODUCTION

The stable isotopes of animal tissue reflect the local dietary input over the time the tissue was synthesized. Different tissues integrate diet over different time scales. Blood or muscle for example, which are commonly used for stable isotope analysis in mammals (Flaherty et al. 2010; Siemers et al. 2011) have turnover rates of some weeks to months (Voigt et al. 2003). However, when research questions require measurement of rapid changes in diet or trophic level, samples with a faster turnover rate such as exhaled breath or faeces are required. Collection of both breath and faeces is relatively easy, cost effective and non-invasive, but so far only a single element (carbon) can be measured in breath (Voigt 2009).

Faeces are a good candidate for stable isotope analysis with the aim of short-term diet investigation, because i. they contain remains of the recent diet (Painter et al. 2009); ii. capturing of animals is not necessarily required as faeces can be collected from below roosting sites; and iii. stable isotopes of multiple elements can be measured in a single sample. Bat ecologists often use faeces for visual (reviewed by Vaughan 1997; Safi & Kerth 2004) or less frequently, molecular (Bohmann et al. 2011) identification of prey items. Stable (Wurster et al. 2010) or radioisotopes (Johnston et al. 2010) in old bat guano are used as paleoclimate records. Only a few studies have conducted stable isotope analysis on bat faeces to investigate ecology (DesMarais et al. 1980), habitat use (Sullivan et al. 2006) and diet (Painter et al. 2009).

The lack of controlled experiments limits the application of faecal stable isotopes in ecology of small mammals. Animal isotopic ecology is still lacking experimental estimations of isotopic diet-sample differences ($\Delta$) (Wolf et al. 2009), i.e. the difference in the isotopic content between the diet and the sample used to estimate it (also known as fractionation factor or isotopic discrimination). A difference between diet and faeces is related to biochemical pathways during digestion or varying digestibility of food components with different isotope values (Hwang et al. 2007). Ideally, the $\Delta$ value would be calculated using the food consumed and the faeces egested by the animal. However, the exact diet of the study animal in the field is often unknown or unavailable for analysis. Values of $\Delta$ can vary among different biomaterials of the same individual (Borrell et al. 2012). Thus, to reconstruct diet from stable isotopes, use isotope mixing models, or compare results of different studies, it is essential to know the $\Delta$ value for the specific biomaterials (Borrell et al. 2012; Caut et al. 2009). Also, for refined time-scale diet investigation, the turnover rate of the isotopic signature of the biomaterial is required to assign the diet to correct time.

Stable isotope values of different elements provide different information. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes, commonly used in bat studies, give information about the
diet, feeding habitat and trophic level of species (Fry 2008). They also indicate whether diet originates from agricultural areas (Hebert & Wassenaar 2005) and stable sulphur isotope ($\delta^{34}$S) encodes the salinity of the feeding habitat (e.g., Fry & Chumchal 2011). Despite its potential, $\delta^{34}$S is seldom used in mammal ecology.

Our aim was to explore the potential of faeces as samples for stable isotope analysis, including $\delta^{34}$S. We performed a diet switch experiment with captive bats, using isotopically different diets to simulate changes that could occur in nature when an animal switches diet or feeding habitat. Since $\delta^{15}$N values are indicative of trophic level (Fry 2008), by changing dietary $\delta^{15}$N values, we simulated prey items of different trophic levels. Due to differences in photosynthetic pathways, C$_3$ and C$_4$ plants differ in $\delta^{13}$C values (Marshall et al. 2007). By varying dietary $\delta^{13}$C, we modeled changes that could occur when the animal is changing feeding habitats, e.g., from those cultivated with C$_4$ plants areas (e.g., Maize) to forest or other habitats with C$_3$ plants (temperate regions). Aquatic and terrestrial insects differ in $\delta^{13}$C and $\delta^{15}$N values (Paetzold et al. 2005; Raikow et al. 2011), thus by changing the bats’ diet in these values, we simulated a shift from feeding on terrestrial to freshwater insects. Values of $\delta^{34}$S are related to salinity and distance from the sea (Zazzo et al. 2011), so with a change in dietary $\delta^{34}$S, we simulated changes that could occur when an animal is feeding at different distances from the sea.

The objectives of the experiment were to: (i) estimate the time after which a dietary switch is reflected in faeces (turnover rate), and (ii) test how accurately faecal stable isotope values represent dietary isotope values. For this purpose we calculated the diet-faeces isotopic differences ($\Delta_{\text{diet-faeces}}$). To obtain an impression of how applicable our results would be across species, we tested whether the faecal stable isotope values, the turnover rates and the $\Delta_{\text{diet-faeces}}$ values differed between two phylogenetically distant species: Myotis myotis (Borkhausen, 1797) (greater mouse-eared bats) and Rhinolophus ferrumequinum (Schreber, 1774) (greater horseshoe bats). We measured faecal $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S values prior to, during and after the diet switches. We expected isotopic signature in faeces to provide accurate information on recently consumed food.

**EXPERIMENTAL**

**Diet**
Young instars of mealworms (larval stages of the beetle Tenebrio molitor Linnaeus, 1758) were split into two groups. They were kept at room temperature and fed for one month prior to the experiment, either with commercially available tin-canned tuna (Thunfischfilets, EDEKA Zentrale AG & Co. KG, New-York-Ring 6, 22297, Hamburg, Germany) or with cereals...
(Matzinger Vollkornflocken mit Gemüse, Nestlé Purina, PetCare Deutschland GmbH, Albert-Latz-Straße 6, 53879, Euskirchen, Germany). Both were supplemented with fruits and vegetables (apples, carrots, salad). The cereal diet was expected to contain lower $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values than the tuna diet (Nardoto et al. 2006). We thus refer to the tuna-fed and cereals-fed mealworms as heavy-labelled and light-labelled mealworms, respectively. Shortly before the experiment started, the mealworms were supplemented with additives containing essential nutrients, including vitamins and minerals (Nutri-Cal, Albrecht GmbH, Hauptstr. 6-8, 88326, Aulendorf, Germany and Korvimin ZVT + Reptil, WDT eG, Siemensstr. 14, 30827, Garbsen, Germany).

**Bats**

We used two phylogenetically distant species (Teeling et al. 2005) that occur sympatrically but differ in their trophic ecology and resource use. *Myotis myotis* preys on ground arthropods (mainly carabid beetles) that it detects by the rustling sounds and gleans from accessible ground surfaces (Arlettaz 1996). *Rhinolophus ferrumequinum* feeds predominantly on large flying moths and beetles (mainly Scarabaeoidea), which it detects by wing movement that is encoded in the echoes of echolocation calls (Kober & Schnitzler 1990; Jones 1990). Different sensory access to prey leads to differences in trophic level; the diet of *M. myotis* is dominated by predatory arthropods (Siemers et al. 2011), whereas herbivorous insects dominate the diet of *R. ferrumequinum* (Jones 1990).

Eight male captive bats were used for the feeding experiment: four *R. ferrumequinum* (weight: 17.9 - 19.1 g) and four *M. myotis* (weight: 29.5 - 30.3 g) individuals that were kept at 22-23 °C and in 60-70% relative humidity. The *R. ferrumequinum* individuals were kept together in a flight cage (length $\times$ width $\times$ height: 2.4 $\times$ 1.2 $\times$ 2 m), but fed in separate small cages (three in 0.3 $\times$ 0.3 $\times$ 0.3 m and one in 0.6 $\times$ 0.45 $\times$ 0.45 m) to enable individualized faeces collection. The individuals remained in the feeding cages for about 2-3 hours from the feeding time, after which the faeces were collected. The *M. myotis* individuals were kept together in a flight cage (2.4 $\times$ 2 $\times$ 2 m), but fed in separate, small woven boxes (0.15 $\times$ 0.15 $\times$ 0.15 m to 0.4 $\times$ 0.25 $\times$ 0.2 m), where they remained for about 2-3 hours from the feeding time until the faeces were collected. Each species was fed at the beginning of the dark phase of the daily photoperiodic cycle.

**Experimental protocol**

Diet was controlled and faeces were collected daily for a duration of 12 days. Each *M. myotis* individual was offered 7-8 g mealworms per day and each *R. ferrumequinum* individual 5-6 g, but they usually consumed less. Water was provided *ad libitum* to all bats. On days 1 and 2 of the experiment, bats were fed their usual diet of light-labelled mealworms. On day 3 we switched
their diet to the heavy-labelled mealworms, and they were fed this diet for 7 consecutive days. On day 10 we switched their diet back to the starting diet of light-labelled mealworms. Bats remained on this diet until the end of the experiment (days 10 through to 12).

**Faecal Samples**
The faecal samples were left in open Eppendorf tubes to dry at room temperature before being stored in a freezer (-30 °C) until the analysis. The samples from one specimen of each species were excluded from the analyses, because these two bats showed an aversion to heavy-labelled mealworms, and they produced negligible amounts of faeces on the days fed when they were fed with these mealworms. At least one faecal sample was analysed per day, per individual bat. Thus, stable isotope analysis was conducted on a total of 72 faecal pellets.

**Stable isotope analysis**
Lipids tend to be more depleted in $^{13}$C compared to tissues (Tieszen et al. 1983). Mealworms have a high lipid content (Jones et al. 1972), which could lead to a bias in the results if the signature of the lipids rather than the rest of the organism is measured. To avoid this bias, we removed lipids from both types of mealworms by rinsing them twice with a 2:1 chloroform:methanol solution. Because we had had no information about the lipid content of faeces, we had tested, in a pilot study, if a lipid extraction would be necessary. We used faecal samples (homogenized subsamples from about 10 faeces each) from *M. myotis* and *M. daubentonii*. Lipids were extracted with a 2:1 chloroform:methanol solution from three samples per species and both these and three untreated faecal samples were analysed for $\delta^{13}$C. There was no significant difference in the $\delta^{13}$C values of samples after lipid extraction and untreated samples (Mann-Whitney Test, $p=0.75$, own unpublished data). Based in this piece of evidence, we proceeded without extracting lipids from faecal samples in the current experiment. Dried and powdered sub-samples of approximately 1.5 mg mealworms (homogenized sample from 3-4 mealworms) and 1.3 mg faeces were weighed in small tin cups to the nearest 0.001 mg, using a micro-analytical balance. Samples were then combusted in a vario Micro cube elemental analyser (Elementar, Analysensysteme GmbH, Hanau, Germany) at the Limnological Institute, University of Konstanz, Germany. The resulting CO$_2$, N$_2$ and SO$_2$ were separated by gas chromatography and inserted into a Micromass Isoprime isotope ratio mass spectrometer (IRMS) (Isoprime Ltd, Isoprime House, Earl Road, Cheadle Hulme, Cheadle, SK8 6PT, UK), for determination of $^{13}$C/$^{12}$C, $^{15}$N/$^{14}$N and $^{34}$S/$^{32}$S ratios. Measurements are reported in $\delta$-notation ($\delta^{13}$C, $\delta^{15}$N, $\delta^{34}$S) in parts per thousand deviations (‰), where $\delta = 1000 \times (R_{sample}/R_{standard}-1)$ % relative to the following standards: Pee Dee Belemnite (PDB) for carbon, atmospheric N$_2$ for nitrogen and sulfanilamide calibrated and
traceable to NBS-127 (barium sulfate, $\delta^{34}\text{S} = +20.3\%$) for sulphur. The ratio (R) of heavy/light isotopes is calculated as: $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$ and $^{34}\text{S}/^{32}\text{S}$. Two sulfanilamide (Iso-prime internal standards) and two casein samples were used as a laboratory standard for every 8 unknowns in sequence. Data obtained with internal laboratory standards indicated measurement errors (SD) of ± 0.05‰, 0.15‰ and 0.05‰ for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$, respectively.

**Calculation of diet-faeces isotopic differences ($\Delta_{\text{diet-faeces}}$)**

The diet-faeces isotopic difference, $\Delta_{\text{diet-faeces}}$ or $\Delta$, was calculated for each element as:

$$\Delta X = \delta X_{\text{faeces}} - \delta X_{\text{diet}}$$

where $X$ is: $^{13}\text{C}$, $^{15}\text{N}$ or $^{34}\text{S}$.

We calculated the $\Delta_{\text{diet-faeces}}$ values for each faeces separately, by subtracting the mean $\delta X$ for the corresponding diet (heavy or light-labelled mealworms) from the faeces stable isotope value. To calculate the mean values for light-labelled diet for *R. ferrumequinum*, we excluded one faeces produced on the first day after the switch back to the light-labelled diet, because it appeared to still have the heavy-labelled signature and we wanted to ensure the calculation of the $\Delta$ value corresponded to each diet separately.

**Statistical Analyses**

The data did not originate from a normal distribution, so we used only non-parametric tests. We compared the isotopic signature of light to the heavy-labelled mealworms with Mann-Whitney U test (five mealworm samples per group). To avoid pseudoreplication and ensure a balanced design, stable isotope values of one randomly selected faeces per day and individual were included in the dataset for statistical analyses of faecal samples. We tested for differences between stable isotope values of the diet and the respective faeces produced with the same diet, i.e. we tested whether $\Delta$ was statistically significant, using Mann-Whitney U test.

We were interested in how diet change is reflected in repeated samples of individual bats. To account for repeated measures while retaining a high statistical power, we used non-parametric longitudinal models for factorial experiments (Brunner et al. 2002; Noguchi et al. 2012a). For measurements of each isotope, we estimated a non-parametric model with both one whole plot factor (bat species) and one subplot which is the time factor (day of the experiment). The isotope values of each individual on different days were treated as measurement repeats. We computed a non-parametric ANOVA-type statistic (ATS), which was developed for use with small sample sizes (Noguchi et al. 2012a). Besides testing for the significance of each factor and their interaction, we also tested the null hypothesis that isotope values on days 3 to 9, when heavy-labelled mealworms were fed, did not exceed isotope values on the other days, when bats were
fed their usual light-labelled diet. This was computed with the following time pattern vector shaped as a boxcar function (the same pattern was assumed for both species, respectively):

\[ t = [1, 1, 2, 2, 2, 2, 2, 2, 1, 1, 1] \]

Further details of this method can be found in Brunner et al. (2002) and Noguchi et al. (2012a,b). With the same procedure, we tested for significant differences in \( \Delta \) values among the days of the experiment and between species, and tested whether \( \Delta \) values of the heavy-labelled diet exceeded those of the light-labelled diet. The analyses were performed using the nparLD package v. 2.0 (Noguchi et al. 2012b) on R version 2.15.0 (R). No data points were excluded for this analysis.

**RESULTS**

**Isotopic signature of faeces - Turnover rate**

As expected, heavy-labelled mealworms exceeded light-labelled mealworms for all stable isotope values analysed (Table 1). The \( \delta^{13} \)C, \( \delta^{15} \)N and \( \delta^{34} \)S values were significantly different between the two types of mealworms (Mann-Whitney U = 15, n = 10, p = 0.008). Thus, the two mealworm types were, due to their different isotopic values, well suited as food in our diet-switch experiment. The C/N mass ratio (mean±SD) of the mealworms (4.334±1.025) was similar to that of the faeces (4.239±0.682 for *M. myotis*, and 4.609±0.367 for *R. ferrumequinum*).

When the bats were fed with differently labelled food, they egested faeces with the new isotopic signature on the same day the diet switch was conducted, after 2-3 hours, when the faeces were collected (Fig. 1). The content of stable isotopes in the faeces differed among the days of the experiment, i.e. the effect of time was highly significant for all three isotopes (ANOVA-type statistic, ATS, p < 10^-5; Table 2). There was no significant difference between species in any of the stable isotope values (ATS with Box modification, p ≥0.084; Table 2), although a marginally non-significant difference was apparent in \( \delta^{15} \)N values between species (p=0.084; Table 2). There was no difference between species in the manner of isotopic signature change over time, i.e. we found no statistically significant interaction between species and time (p ≥0.418; Table 2). A further test revealed which days the faecal stable isotopes differed. Namely, the effect of time was significant, because the values of faecal stable isotopes on days with heavy-labelled diet exceeded those on the remaining days with light-labelled diet. In other words, the hypothesis of a time pattern shaped like a boxcar function could be confirmed for both species and all stable isotopes (p ≤0.013; Table 2). The boxcar-shaped time pattern is also visible in Figure 1.
**Diet-faeces isotopic differences ($\Delta_{\text{diet-faeces}}$)**

The mean $\Delta_{\text{diet-faeces}}$ values from all individuals (both species and both diets) were: $+1.47 \pm 1.51\%$ for $^{15}\text{N}$; $-0.11 \pm 0.80\%$ for $^{13}\text{C}$; and $+0.74 \pm 1.10\%$ for $^{34}\text{S}$ (Table 1). The isotopic signature did not differ between faeces and diet; in other words $\Delta$ did not differ significantly from zero in almost any case, except for $^{15}\text{N}$ in faeces of *M. myotis* (Table 1) which showed a statistically significant $\Delta$ for both light and heavy-labelled diet (Mann-Whitney $U = 89$, $p = 0.015$ and $U = 70$, $p = 0.002$ respectively). Faeces of *M. myotis* had $\delta^{15}\text{N}$ values greater than heavy-labelled mealworms by $+2.34 \pm 2.17\%$, and light-labelled mealworms by $+1.81 \pm 1.28\%$. The content of the other two stable isotopes in *M. myotis* and the content of any stable isotopes in *R. ferrumequinum* did not differ significantly between the diet and the faeces (Mann-Whitney test, all $p > 0.05$ and Table 1). The figure 2 shows the boxplots of the $\Delta$ values (calculated from the single $\Delta$ value of each faeces) per element, per diet and per species.

![Figure 1](image)

**Fig. 1** Faecal stable isotope ratios in each day of the experiment per species (filled symbols: *M. myotis*, open symbols: *R. ferrumequinum*), (a) $\delta^{13}\text{C}$, (b) $\delta^{15}\text{N}$, (c) $\delta^{34}\text{S}$. The isotope values of light and heavy labelled mealworms (grey background) are shown in the right panels. The heavy-labelled mealworms were fed on days three to nine (grey background) and light-labelled mealworms on the remaining days.
The $\Delta$ values did not differ between species for any of the elements ($p \geq 0.078$; Table 3). The lower $p$-value for $\Delta^{15}$N (0.078) is related to the above-mentioned significant enrichment in $\delta^{15}$N of *M. myotis*, which was not found in *R. ferrumequinum*. Only $\Delta^{13}$C differed according to the day of the experiment, irrespective of diet ($p = 6.9 \times 10^{-5}$) (Table 3). Within the species, $\Delta^{13}$C varied significantly among days in *M. myotis* (ATS = 6.91, df = 1.86, $p = 0.001$), but not in *R. ferrumequinum* (ATS = 2.79, df = 1.46, $p = 0.079$). This daily variation was not caused by the difference in the $\Delta$ values of the heavy-labelled and light-labelled diet. The $\Delta^{13}$C of heavy-labelled mealworms did not exceed that of the light-labelled mealworms and the time pattern shaped like a boxcar function was not statistically significant for either of the species ($p \geq 0.166$; Table 3). Rather, $\Delta^{13}$C increased slightly throughout the course of the experiment.

![Fig. 2](image-url) Diet-faeces isotopic differences ($\Delta_{\text{diet-faeces}}$ or $\Delta$) for stable isotopes of (a) carbon ($\Delta^{13}$C), (b) nitrogen ($\Delta^{15}$N) and (c) sulphur ($\Delta^{34}$S), for each diet (left) and pooled from both diets, light-labelled mealworms (ll) and heavy-labelled mealworms (hl), per each species, *M. myotis* (M.m.) and *R. ferrumequinum* (R.f.) (right).
DISCUSSION

Isotopic signature of faeces

Using a triple isotopic experiment, we showed that faecal stable isotopes reflect rapidly the most recent diet. Faeces are often used in bat ecology to examine prey remains either visually or with molecular methods, in order to reveal the taxonomic identity of the prey. Stable isotopes provide information on preys’ ecological (habitat, trophic level) and geographic origin, as well as their relative contribution in the diet, when used in mixing models (Phillips 2012). Each method provides different and valuable information. These methods are complementary and should ideally be used together to give a broader picture of resource use or be selected according to the research aim.

A high C/N mass ratio can be related to high lipid content in a particular tissue (Post et al. 2007). The C/N mass ratio of the bat faeces of the feeding experiment indicates low lipid content. Lipid-extracted mealworm samples had a very similar C/N mass ratio to the faeces. This confirms that lipids do not have to be extracted from the faeces before the stable isotope analysis.

We supplied the bats with isotopically significantly different diets, to ensure the assignment of the faeces to the respective diet. However, such large differences in dietary signatures might not always occur in the wild. In this case, multiple elements can provide more detailed information and reveal additional aspects of trophic ecology from a single sample. A three-isotope approach should be favored, as it can reveal a diet heterogeneity that could be overlooked with a dual-isotope approach (Moreno et al. 2010). Stable sulphur isotope is rarely used in animal ecology (but see Cryan et al. 2012). Our study is one of the few on mammals (e.g., Cryan et al. 2012) adding $^{34}$S to the commonly used $^{13}$C and $^{15}$N isotopes. Marine sulphur contains more of the heavy isotope and is transferred to terrestrial systems with a spray effect (Wadleigh et al. 1996). Values of $\delta^{34}$S encode the distance of feeding habitat from the sea and this makes it suited to determine food origins and to track animal movements.

The isotopic signature of the faeces did not change much during the days when the bats were eating the same diet. The minor fluctuations were probably caused by the presence of varying amounts of food particles with older isotopic signature. There was an increasing tendency in $\delta^{13}$C values, although the time pattern in the shape of a boxcar function was statistically significant. This could be possibly attributed to slightly increasing $\delta^{13}$C values in mealworms. The random variation in the proportion of cereals (corn and wheat) in the mixture fed to the light-labelled mealworms might have resulted in an increased $\delta^{13}$C signature. The variation in $\delta^{13}$C values of mealworms was, indeed, higher than for $\delta^{15}$N and $\delta^{34}$S (Fig. 1). The increase in faeces $\delta^{13}$C values might also be related to the increase of $\Delta^{13}$C throughout the experiment. Physiological
processes during digestion could also explain the faeces $\delta^{13}C$ and $\Delta^{13}C$ increase during the last days of the experiment, which would require further study.

**Turnover rate**

The faecal stable isotopes reveal a dietary change within 2-3 hours after the new type of food has been ingested. This was evidenced by the boxcar-shaped time pattern of isotope values in faeces that matched the time pattern of food’s isotope values. Our findings complement those of another study (Stalinski 1994) which found comparably short gut-passage time for ingested food in *M. myotis*: ($t_{90} = 77$ min). Gut passage time in *Eptesicus serotinus* (serotine bat) ranged from 33 min to 32 hours, decreasing with particle size (Robinson & Stebbings 1993). We found the same turnover rate for both species considered in this study. However, this lack of interspecific difference might not be the case for the turnover rates between diet and tissues such as blood and skin (for nectarivorous bats see Voigt & Matt 2004). Therefore, when the purpose of the study is to compare diets of different species and the turnover rates of tissues from these species are unknown, faeces might be a better choice to estimate diet.

**Diet-faeces isotopic differences**

The $\Delta_{\text{diet-faeces}}$ that we measured can be applied to calculate dietary stable isotope values from the faeces when diets are unknown or unavailable e.g., in the field, where researchers might collect faeces without catching the animals. Except for $\delta^{15}N$ in *M. myotis*, we did not find any significant differences in stable isotope values between diet and faeces. Stable carbon and sulphur isotopes showed no significant difference between diet and faeces. The difference in $\Delta^{15}N$ between species was a non-significant trend in the longitudinal model that accounted for the repeated measures. Different proportions of digested parts of the mealworms in the faeces are probably responsible for the trend of difference we found in $\Delta^{15}N$ between the two species. The relatively lower (for $^{15}N$) and almost negligible (for $^{13}C$ and $^{34}S$) $\Delta$ values, which were rather stable among the days of the experiment, species and diets, make faeces a reliable source of dietary information. When reconstructing diet from faecal stable isotope values in insectivorous bats, $\Delta^{15}N$ should be included in the calculations, while $\Delta^{13}C$ and $\Delta^{34}S$ can be ignored without any loss of accuracy.

The $\Delta$ value calculated for $^{13}C$ between diet (mealworms) and hair of *M. myotis* (Siemers et al. 2011) was higher ($+3.58 \pm 0.28\%$) than what we found ($-0.22 \pm 0.90\%$, average for *M. myotis* from both diets). The obvious explanation is that different biochemical processes are involved in the formation of hair and digestion. The $\Delta^{15}N$ from the previous study (Siemers et al. 2011) ($+2.58 \pm 0.09\%$) is almost similar to the value we found ($+2.12 \pm 1.85\%$: mean±sd for *M. myotis* faeces across diets).
The $\Delta_{\text{diet-faeces}}$ we calculated, did not differ between the two species belonging to different suborders of Chiroptera (Teeling et al. 2005). Thus our data could be applied to other insectivorous bats or other small mammals with similar diets, bearing in mind that the $\Delta_{\text{diet-faeces}}$ of species with a very different body size may differ from the ones we report. Additional factors that can affect the $\Delta$ values should be considered, e.g., food protein content (McCutchan et al. 2003), diet quality and isotopic signature, age, and condition of the organism (reviewed by Caut et al. 2009), or for $^{15}$N, water or nutritional stress (Kelly 2000).

Faeces are excreted materials of an ingested diet. A disadvantage of using faeces for diet investigations could be that they do not provide information on assimilated diet. However, our study indicates that faeces better represent the isotopic signature of the food that was ingested than tissues do. Therefore, faeces or tissue samples should be selected dependent on whether the study aims to investigate the diet, or the assimilated part of it.

**Further applications**

Our results can be applied to study topics such as: i) short-term differences of diet in bat and other mammalian species, ii) individual or population dietary specialization, iii) opportunistic behaviour of individuals, populations or species, iv) habitat choice, and v) movements among different habitats. Faecal stable isotopes could be implemented to monitor pollution and investigate whether animals feed on sites with anthropogenic impact. Values of $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S differ between organic material and sewage (Spies et al. 1989) and these tracers have already been used as tracers of pollution in marine systems (Van Dover et al. 1992). Bat populations in Europe and North America declined in the past century, partly due to the extensive use of pesticides in agriculture (Kunz et al. 1977). Monitoring of resource use in agricultural areas using stable isotopes may assist in developing conservation plans for bats and other small mammals.

**CONCLUSIONS**

We found that $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S values in faeces of insectivorous bats reflect the signature of the recently consumed diet within 2-3 hours after ingestion. The turnover rate and the $\Delta$ values did not differ between two distantly-related bat species fed the same diet. Thus, these results may apply to other insectivorous mammals. The $\Delta_{\text{diet-faeces}}$ values from our study should be carefully applied to species with different diets. Advantages of faeces are the possibility for non-invasive sampling and their suitability for answering questions concerning short-term diet or habitat variation. In combination with methods, such as visual identification or molecular analysis of prey items in faeces, stable isotopes analysis can provide additional information and be useful for monitoring.
ACKNOWLEDGEMENTS

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**Table 1.** The stable isotope values ($\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S) (mean ± standard deviation: s.d.) of the two types of mealworms and faeces for the two bat species (*M. myotis, R. ferrumequinum*) and the relevant diet-faeces isotopic differences ($\Delta_{\text{diet-faeces}}$ or $\Delta$) per species and diet as well as their overall means from both species and diets. n= number of samples analysed and in brackets number of individual bats. * A single data point was excluded from the calculation of the $\Delta$ values (n=14, in this case).

<table>
<thead>
<tr>
<th>Diet</th>
<th>samples/species</th>
<th>n</th>
<th>$\delta^{15}$N (mean ± sd)</th>
<th>$\delta^{13}$C (mean ± sd)</th>
<th>$\delta^{34}$S (mean ± sd)</th>
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<tbody>
<tr>
<td>light-labelled</td>
<td>mealworms</td>
<td>5</td>
<td>+5.31 ± 0.63</td>
<td>-24.54 ± 0.76</td>
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<tr>
<td></td>
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<td>+3.98 ± 0.89</td>
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<td></td>
<td><em>R. ferrumequinum</em></td>
<td>15* (3)</td>
<td>+6.23 ± 1.63</td>
<td>-24.54 ± 1.12</td>
<td>+4.16 ± 1.54</td>
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<td></td>
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<th>samples</th>
<th>n</th>
<th>$\Delta^{15}$N (mean ± sd)</th>
<th>$\Delta^{13}$C (mean ± sd)</th>
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<td><em>R. ferrumequinum</em></td>
<td>21 (3)</td>
<td>0.97±0.45</td>
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Table 2. Results of non-parametric longitudinal models for time, species and time-species interaction effects on the stable isotope values of $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S of the faeces collected from the two bat species (*M. myotis, R. ferrumequinum*), as well as a test of higher levels of stable isotope values on days with heavy-labelled diet. These higher levels are evidenced by the statistically significant boxcar-shaped time pattern.

<table>
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<td></td>
<td>p</td>
<td>$&lt; 10^{-5}$</td>
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<td></td>
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Table 3. Results of non-parametric longitudinal models for time, species and time-species interaction effects on the diet–faeces isotopic differences ($\Delta_{\text{diet-faeces}}$ or $\Delta$) of $^{13}\text{C}$ ($\Delta^{13}\text{C}$), $^{15}\text{N}$ ($\Delta^{15}\text{N}$) and $^{34}\text{S}$ ($\Delta^{34}\text{S}$) of the faeces collected from the two bat species (*M. myotis*, *R. ferrumequinum*) during the feeding experiment. Here we tested the boxcar-shaped time pattern only for $^{13}\text{C}$, because in other elements $\Delta$ value did not significantly change among days (time effect was not significant). Non-significant time pattern indicates that change in $\Delta^{13}\text{C}$ was not related to the diet type.

<table>
<thead>
<tr>
<th>Effect</th>
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<th>$\Delta^{15}\text{N}$</th>
<th>$\Delta^{34}\text{S}$</th>
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CHAPTER III

Tracking diet preferences of bats using stable isotope and fatty acid signatures of faeces

Monika My-Y Lam, Dominik Martin-Creuzburg, Karl-Otto Rothhaupt, Kamran Safi, Elizabeth Yohannes, Ioanna Salvarina

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ABSTRACT

Stable isotope and fatty acid signatures of biomaterials can provide important information about the dietary niche of animals. Stable isotope and fatty acid signatures differ between aquatic and terrestrial food webs, and therefore can be used to assess the aquatic and terrestrial contributions to the diets of species. We studied faecal samples of three co-occurring bat species with known differences in feeding preferences. The aim was to assess whether stable isotope and fatty acid signatures of faeces can be used to determine feeding preferences. We hypothesised that faeces stable isotope and fatty acid signatures will reveal the terrestrial, aquatic and mixed feeding niches of *Myotis myotis*, *M. daubentonii*, and *M. mystacinus*, respectively. As predicted, the faeces of *M. myotis* were characterized by higher $\delta^{13}$C values and higher concentrations of linoleic acid and total $\omega$6 polyunsaturated fatty acids (PUFAs), which are typically higher in terrestrial food webs. The faeces of *M. daubentonii* had higher $\delta^{15}$N values and higher concentrations of docosahexaenoic acid and total $\omega$3 PUFAs, characteristic features of aquatic systems. *Myotis mystacinus* faeces had intermediate $\delta^{15}$N values and concentrations of both types of fatty acids. Our results show that analysing stable isotope and/or fatty acid signatures of faeces provides a promising, non-invasive tool to study the feeding ecology of bats and to assess aquatic-terrestrial interactions.
INTRODUCTION

Biochemical tracers, such as stable isotopes and fatty acids, can provide useful information on feeding habits and ecological niches of animals (Ramos & González-Solís 2012). They can help to understand food web complexity, the coexistence of species, and to elucidate mechanisms maintaining species diversity. In addition, identifying an animal’s preferred habitats is crucial for establishing conservation management plans. Since these tracers are different between different habitats, they can be applied to explore species’ flexibility in changing feeding habitats, for example from terrestrial to aquatic. In many areas, aquatic food sources are limited or suffer due to anthropogenic changes and pollution. Biochemical tracers can be also applied to investigate species’ dependence on aquatic resources, and to assess the degree of aquatic subsidies into terrestrial systems and vice versa, which is important for the understanding of the ecosystem functioning.

Bats, among other terrestrial organisms, can depend partially or entirely on aquatic prey for their nutrition. We used collections of bat faeces to investigate whether the stable isotope and fatty acid tracers could discriminate between aquatic and terrestrial feeding preferences in bats. Stable isotope and fatty acid signatures have been analysed in samples derived from the capture of animals to acquire blood, muscle, skin, breath, adipose tissue, liver or the entire carcass (Voigt et al. 2003; Voigt 2009; Melo et al. 2012; McGuire et al. 2013). Collecting these samples is not only laborious, particularly for nocturnal animals, but is also invasive and it should be minimised.

In contrast, collecting faeces is easy and cost-efficient and is usually completely non-invasive when collected, for example, from below bat roosting sites. Faeces have rarely been subjected to stable isotope analysis (but see DesMarais et al. 1980; Sullivan et al. 2006; Painter et al. 2009) and to the best of our knowledge, never to fatty acid analysis for samples from bats or other small mammals. Visual identification of prey remains in bat faeces is common (Brack & Whitaker 2001; Zortéa 2003), but time consuming and requiring expertise in insect identification. Further, it cannot always be used to discriminate the origin of prey (e.g., aquatic or terrestrial). Molecular techniques, which also have been applied to faeces to study the composition of prey species (Bohmann et al. 2011), are more complete but at the cost of being expensive.

Stable isotopes can be used to trace the sources of organic matter to terrestrial or aquatic systems (Phillips & Gregg 2001), as different food webs exhibit different isotopic signatures. Aquatic and terrestrial isotope signatures vary regionally, but within the same region, freshwater biota often have higher stable nitrogen isotope ($\delta^{15}$N) and lower stable carbon isotope ($\delta^{13}$C) signatures than terrestrial biota of comparable trophic levels (Paetzold et al. 2005; Walters et al. 2008; Raikow et al. 2011). Isotopic signatures also differ between freshwater and marine
invertebrates (Keith et al. 1964; France 1994). Stable sulphur isotope values (δ34S) are generally higher in freshwater compared to terrestrial ecosystems (Nehlich et al. 2010), but do not always discriminate freshwater from terrestrial consumers (Privat et al. 2007).

The content and composition of polyunsaturated fatty acids (PUFAs) in organic matter also typically differs between aquatic and terrestrial systems as well as between marine and freshwater sources. Marine invertebrates usually contain higher proportions of ω3 (omega-3, n-3) PUFAs compared to freshwater invertebrates which often have higher proportions of ω6 (omega-6, n-6) PUFAs (Chanmugan et al. 1983). Marine food webs have 5 to 20 fold higher concentrations of ω3 PUFAs than ω6 PUFAs (Olsen 1999). In terrestrial food webs, ω6 PUFAs are more abundant than ω3 PUFAs (Parrish 1999; Fontaneto et al. 2011). Thus, the concentrations of ω3 and ω6 PUFAs and the ratio of ω3/ω6 can be informative for assessing the relative contribution of aquatic and terrestrial food in an organism’s diet. Mammals feeding on aquatic prey have tenfold higher concentration of docosahexaenoic acid (DHA, 22:6n-3, ω3 PUFA) in peritoneal adipose tissue than species feeding on terrestrial diets (European otter, Lutra lutra compared to stone marten, Martes foina and European wild cat, Felis sylvestris) (Koussoroplis et al. 2008). In contrast, linoleic acid (LIN, 18:2n-6, ω6 PUFA) is more concentrated in mammals feeding on terrestrial diets (Koussoroplis et al. 2008). The ratio DHA/LIN has been proposed as a proxy for following changes in terms of aquatic and terrestrial contributions in the diets of carnivorous mammals (Koussoroplis et al. 2008).

The aim of our study was to investigate the suitability of stable isotope and fatty acid signatures from faecal samples to detect aquatic versus terrestrial prey items of different bat species. We expected that differences in these tracers between aquatic and terrestrial prey organisms would be reflected in the stable isotope and fatty acid signatures of faeces from bats feeding on aquatic or terrestrial prey. Earlier, in a diet-switching experiment, we confirmed that bat faecal stable isotopes reflect the signature of the most recent food with a turnover rate of 2-3 hours (Salvarina et al. 2013). We analysed the faeces of three bat species from the genus Myotis inhabiting the same region but with different feeding preferences in terms of aquatic and terrestrial prey. Myotis myotis (greater-mouse eared bat, Borkhausen 1779) has been reported to prey on terrestrial arthropods, especially Carabidae but also on Grillidae, Arachnida, and larvae of Lepidoptera in open areas, fresh cut meadows or forests (Arlettaz 1996). Myotis daubentonii (Daubentons’s bat, Kuhl 1817) is known to hunt over still waters or slow moving rivers and mainly preys on Chironomidae emerging from the water (Vaughan 1997; Encarnação et al. 2010; Krüger et al. 2012). Myotis mystacinus (Whiskered bat, Kuhl 1819), appears to be more flexible in foraging behaviour, is known to hunt in parklands, woodlands and over running water (Bat Conservation Trust 2007), where it mostly feeds on Diptera (Tibulidae, Chironomidae,
Anisopodidae), but these bats have also been reported to consume Arachnida and Lepidoptera (Vaughan 1997; Safi & Kerth 2004).

We predicted that faeces of *M. myotis*, the terrestrial feeder, would display terrestrial signatures, with higher proportions of LIN and total ω6 PUFAs, higher δ¹³C and lower δ¹⁵N values. *Myotis daubentonii*, the aquatic feeder, was expected to have an aquatic signature, i.e. higher proportions of DHA and total ω3 PUFAs, lower δ¹³C and higher δ¹⁵N values. For *M. mystacinus* which feeds both on aquatic and terrestrial insects, we expected an intermediate signature. Finding an aquatic or terrestrial signature for individual *M. mystacinus* faecal pellets would not be surprising, given that they might have been produced by individuals that had consumed more of one prey type than the other.

**MATERIAL AND METHODS**

*Ethic statement*

Sampling was conducted in collaboration with bat conservation organizations active in Konstanz and Kreuzlingen (‘Arbeitsgemeinschaft Fledermausschutz BW e.V.’ and ‘Fledermausschutz Thurgau’, respectively). The species we studied are listed as ‘of least concern’ according to the IUCN red list (IUCN 2013). All samples were collected at privately owned buildings after asking for permission from the owner or manager. No special permissions were required as the animals were not disturbed.

*Sample collection*

Faecal samples were collected in Switzerland and Germany in the vicinity of Lake Constance (Fig. 1). To collect fresh faeces from roosts, we placed a plastic sheet on the floor, underneath the bats, the day before collection. In the end of April on the same day, we collected faeces of *M. myotis* in attics of churches located in Ermatingen and in Lipperswil (both in Switzerland), which are approximately 0.5 km and 6.5 km from Lake Constance, respectively. From Lipperswil we also collected samples from May to June 2011. Faeces of *M. daubentonii* were collected, in May and June 2011, from a hospital attic in Kreuzlingen (Switzerland), approximately 1 km from Lake Constance. Faeces of *M. mystacinus* were collected in May 2011, from behind a shutter on a house in Dingelsdorf, Konstanz (Germany), approximately 0.5 km from Lake Constance. We transported samples to the laboratory and stored them at -80°C until further processing.

We analysed 6 faecal samples for stable isotopes and another 6 for fatty acids per sampling date for each species. The pellets were chosen by selecting the first pellets that forceps touched in
the sample container. A total of 71 samples were analysed for stable isotopes and another 71 for fatty acids (in each case: *M. myotis*: n=29, *M. mystacinus*: n=24 and *M. daubentonii*: n=18).

![Map of faecal sampling locations](https://via.placeholder.com/150)

**Fig. 1** Map of faecal sampling locations (solid circles) for *Myotis myotis* (Ermatingen and Lipperswil), *M. mystacinus* (Dingelsdorf) and *M. daubentonii* (Kreuzlingen). In the top left the broad sampling area is marked in a box (modified from © OpenStreetMap contributors).

**Stable isotope analysis**

Faeces were oven dried at 50°C, ground and 1.5±0.001 mg was weighed in tin capsules on a microbalance (Mettler Toledo Excellence Plus XP6). A sample consisted of one faecal pellet, except in a few cases where two pellets from *M. mystacinus* had to be used due to the small faeces of this species. Stable isotope analyses for nitrogen (δ¹⁵N), carbon (δ¹³C) and sulphur (δ³⁴S), were conducted on the same sample, combusted in a Micro cube (Elementar, Germany) elemental analyser (Limnological Institute, University of Konstanz, Germany). The resulting N₂, CO₂ and SO₂ were separated by gas chromatography and admitted into the inlet of a Micromass (Isoprime, UK) Isoprime isotope ratio mass spectrometer (IRMS) for determination of δ¹⁵N/δ¹⁴N, δ¹³C/δ¹²C and δ³⁴S/δ³²S, respectively. Replicate standards of sulphanilamide (Isoprime internal standards) and casein (source: Elementar Analysensysteme GmbH, Germany) were used as laboratory standards for every 8 unknown samples in sequence. The measurements are reported in δ-notation (δ¹⁵N, δ¹³C, δ³⁴S, respectively) in parts per thousand deviations (%), where δ = 1000 x (Rsample/Rstandard)-1 ‰, relative to atmospheric N₂ for nitrogen, to the Pee Dee Belemnite
(PDB) for carbon, and sulphanilamide calibrated and traceable to NBS-127 (barium sulphate) for sulphur. R= heavy/light isotopes: $^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$, $^{34}\text{S}/^{32}\text{S}$. Internal laboratory standards indicate that our measurement errors (SD) were ± 0.15‰, 0.05‰ and 0.05‰ for $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$, respectively.

**Fatty acid analysis**

The lipids were extracted twice from approximately 10 mg of freeze-dried faecal samples (usually 2-4 pellets) with dichloromethane/methanol (2:1 v/v). The pooled extracts were evaporated to dryness with nitrogen. Fatty acids were transesterified with 3 mol L$^{-1}$ methanolic HCl (60°C, 20 min). Fatty acid methyl esters (FAMEs) were extracted three times with 2 ml iso-hexane. The combined extracts were evaporated to dryness with nitrogen and resuspended in 10 µl iso-hexane. FAMEs were analysed by gas chromatography (GC) on an HP6890. The GC was equipped with a flame ionization detector and a DB-225 (J & W Scientific) capillary column. Details of GC configurations are given elsewhere (Martin-Creuzburg et al. 2009). FAMEs were identified by comparing retention times with that of reference substances (Supelco FAME standard, complemented by 18:1n-9, 18:4n-3, 20:1n-7) and quantified by comparison to internal standards (17:0 ME and 23:0 ME) of known concentrations using multipoint calibration curves determined previously with lipid standards. The identification of fatty acids was verified by analysing mass spectra recorded in selected samples using a gas chromatograph-mass spectrometer (GC-MS; Agilent Technologies, 5975C inert MSD) as described before (Martin-Creuzburg et al. 2009).

For the evaluation of fatty acids, we summed all $\omega$3 and $\omega$6 PUFAs and also calculated the $\omega$3/$\omega$6 ratio. We also considered single $\omega$3 and $\omega$6 PUFAs, i.e., DHA and LIN. The fatty acid data were evaluated and represented as percentages of total fatty acids present in a sample (%TFA, Total Fatty Acid).

**Statistical analyses**

We checked if the stable isotope ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\delta^{34}\text{S}$) and fatty acid ($\omega$3, $\omega$6, DHA, LIN) data deviated significantly from a normal distribution (Shapiro-Wilk test, $p>0.05$). For Gaussian distributed data or data that could be transformed into a normal distribution we applied parametric tests (ANOVA), else we applied non-parametric tests (Kruskal-Wallis). To assess whether the parameters (i.e., stable isotopes, $\omega$3, $\omega$6, $\omega$3/$\omega$6, DHA, LIN and DHA/LIN values) were different between the two populations of *M. myotis* at the near versus far from the lake locations (Ermatingen and Lipperswil, respectively) we compared the values of all parameters from the two sites ($n=6$ per site) using t-tests. Since there was no significant differences ($p>0.05$) in any parameters, except $\delta^{34}\text{S}$, the samples were pooled for further analysis.
We used analysis of variance (ANOVA) or the non-parametric Kruskal-Wallis test to investigate differences between the three species (for each parameter separately) and subsequent post-hoc tests (Tukey’s HSD). The tests for the fatty acids were conducted on arcsin-transformed values of the proportions. The ANOVAs were done on i) a dataset with samples summed over all the dates for each species and ii) a dataset with only the samples collected during the same 2 weeks (M. myotis: 25th May, M. mystacinus: 18th May and 31st May, M. daubentonii: 17th May). We chose to compare only these samples, as they were collected on days close to each other and because we could not collect samples on the same day for logistic reasons.

We used general linear models (GLMs) with species and sampling date as factorial covariates to determine whether any explained the variation in each parameter. To investigate the ability to assign the samples to the correct species based on the stable isotope or fatty acid values we performed a linear discriminant function analysis separately for the stable isotope (δ¹⁵N, δ¹³C, δ³⁴S) and fatty acid data (ω3, ω6, ω3/ω6). All statistical analyses were performed using R version 2.15.2 (R). The package ‘sciplot’ (Morales 2010) was used for plotting means and standard errors, the package ‘pgirmess’ for the multiple comparisons after the Kruskal-Wallis tests and the package ‘MASS’ (Venables & Ripley 2002) was used for the linear discriminant analysis.

RESULTS

Stable isotopes

The values of δ¹⁵N were different between all three species (Kruskal-Wallis, df=2, X²=48.31, p<0.001) (Table 1). Myotis daubentonii (mean±se: 9.10±1.44‰) faeces were more enriched in δ¹⁵N than M. myotis (mean±se: 1.87±1.32‰), while M. mystacinus had intermediate values (mean±se: 5.69±1.99‰) (Fig. 2A). The differences in δ¹³C were less pronounced (Fig. 2A, Fig. 2B). Myotis myotis and M. mystacinus differed in their δ¹³C values (ANOVA, post-hoc test, F₂,₆₈=8.37, p<0.001), while the δ¹³C values for M. daubentonii did not differ from M. myotis (ANOVA, post-hoc test, F₂,₆₈=8.37, p=0.097), nor from M. mystacinus (ANOVA, post-hoc test, F₂,₆₈=8.37, p=0.262) (Table 1). The values of δ³⁴S (Fig. 2B) were different between M. myotis and the other two species (Kruskal-Wallis, df=2, X²=54.03, p<0.001) (Table 1).

A strong temporal change occurred in the stable isotope values in the faeces of M. daubentonii (ANOVAs, for all isotopic elements: p<0.005), with an increasing trend in δ¹³C and δ³⁴S (Fig. 3, Table 2). The temporal differences in the isotopic values of the faeces of M. myotis were more pronounced for δ¹³C (ANOVA, F₃,₂₅=21.03, p<0.001) and for δ¹⁵N (ANOVA, F₃,₂₄=13.85, p<0.001) showing a decreasing trend (Fig. 3, Table 2). A weak temporal change (ANOVA, F₃,₁₉=5.03, p=0.009) was noted in δ¹³C values in the faeces of M. mystacinus (Fig. 3, Table 2).
When we compared $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S between the species using only the samples collected during the same period (middle of May), all values for all species pairs were different (ANOVAs for $\delta^{15}$N and $\delta^{13}$C, Kruskal-Wallis for $\delta^{34}$S, p<0.001), except $\delta^{34}$S values between *M. Mystacinus* and *M. daubentonii* (Table 1). The GLMs showed that variation in the $\delta^{13}$C and $\delta^{15}$N values was explained both by species identity (for $\delta^{13}$C: *M. myotis* and *M. daubentonii*: p<0.001 and for $\delta^{15}$N: for all species p<0.001) and date (for both isotopes p<0.001) (Table 3). The variation in $\delta^{34}$S was explained by species (*M. myotis*: p<0.001 and *M. mystacinus*: p<0.001) while date was not significant (p=0.094) (Table 3).

**Table 1.** Results of the ANOVAs and post-hoc tests or Kruskal-Wallis for the comparison of the stable isotope values between the species, using i) all the samples and ii) the samples collected in the middle of May only.

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<td>&lt;0.001</td>
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<td>$X^2$=48.31</td>
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</tr>
<tr>
<td>test</td>
<td>Kruskal-Wallis</td>
<td>ANOVA</td>
</tr>
</tbody>
</table>

*myo: Myotis myotis, mys: M. mystacinus, dau: M. daubentonii.* The statistically significant values are indicated in bold.

**Fig. 2** Plot of stable isotope values **A.** $\delta^{15}$N versus $\delta^{13}$C and **B.** $\delta^{34}$S versus $\delta^{13}$C for Myotis myotis, *M. mystacinus* and *M. daubentonii*. Values indicate that each species occupied a different isotopic niche. The population of *M. myotis* (Ermatingen) (open circles), which roosted closer to water, occupied the same niche as the population farther away (full circles).
Chapter III  
**Stable isotopes and fatty acids in bats’ faeces**

**Fig. 3** Temporal patterns (means ± SE) in A. δ^{15}N, B. δ^{13}C, C. δ^{34}S, D. ω3 PUFAs and, E. ω6 PUFAs values for *Myotis myotis*, *M. daubentonii* and *M. mystacinus*. Lip: Lipperswil, Erm: Ermatingen.

**Fatty acids**

We found differences for almost all pair-wise comparisons of the concentrations of total ω3 and ω6 PUFAs, DHA, LIN, and the ratios DHA/LIN and ω3/ω6 (Table 4). Only the concentration of DHA and the ω3/ω6 ratio did not differ between *M. daubentonii* and *M. mystacinus* and the concentration of LIN was not different between *M. myotis* and *M. daubentonii* (Table 4). The faeces of *M. daubentonii* were characterized by an almost threefold higher concentration of ω3 PUFAs relative to *M. myotis* (Fig. 4A, Fig. 5). In contrast, the concentrations of ω6 PUFAs in the faeces of *M. myotis* were higher (by 10.28±1.27% TFA) than in *M. daubentonii* (Fig. 4B, Fig. 5). Both, ω3 and ω6 PUFA concentrations in the faeces of *M. mystacinus* were intermediate to the other two species (ω3: 17.43±1.85% TFA and ω6: 19.54 ±3.69% TFA) (Fig. 4A, Fig. 4B, Fig. 5).

The single PUFAs, especially DHA and to a lesser extent LIN, showed similar patterns to the total ω3 and ω6 PUFAs, respectively. DHA concentrations were higher in the faeces of *M. daubentonii* than in the faeces of *M. myotis* (mean±se: 0.31±0.05% TFA vs. 0.07±0.05% TFA, respectively) while the opposite was the case for LIN (0.54±0.04% TFA vs. 4.30±3.64% TFA, respectively). *Myotis mystacinus* had intermediate concentrations of DHA (0.22±0.03% TFA) and of LIN (0.97±0.14% TFA).
Table 2. Results of the ANOVAs and Kruskal-Wallis tests for the comparison of the stable isotope and fatty acid values between the different sampling dates for each species.

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<tr>
<td>test</td>
<td>Kruskal-Wallis</td>
<td>ANOVA</td>
<td>ANOVA</td>
</tr>
<tr>
<td>LIN</td>
<td>p value</td>
<td>0.271</td>
<td>0.093</td>
</tr>
<tr>
<td>statistics</td>
<td>df=3, X²=3.91</td>
<td>df=3, X²=6.43</td>
<td>F₂,₁₅ =-0.419</td>
</tr>
<tr>
<td>test</td>
<td>Kruskal-Wallis</td>
<td>Kruskal-Wallis</td>
<td>ANOVA</td>
</tr>
<tr>
<td>DHA/LIN</td>
<td>p value</td>
<td>0.005</td>
<td>0.119</td>
</tr>
<tr>
<td>statistics</td>
<td>df=3, X²=12.91</td>
<td>df=3, X²=5.86</td>
<td>df=2, X²=2.47</td>
</tr>
<tr>
<td>test</td>
<td>Kruskal-Wallis</td>
<td>Kruskal-Wallis</td>
<td>ANOVA</td>
</tr>
</tbody>
</table>

The statistically significant values are indicated in bold.

A lower ω₃/ω₆ ratio indicated greater consumption of terrestrial prey (M. daubentonii>M. mystacinus>M. myotis). The ω₃/ω₆ ratio was more than four times higher in M. daubentonii than in M. myotis and a similar trend occurred for the DHA/LIN ratio (Fig. 4C). Interestingly, the ω₃/ω₆ and DHA/LIN ratios from faeces of M. mystacinus were relatively balanced (ω₃/ω₆=1.14±0.06 and DHA/LIN=0.28±0.03).

Concentrations of ω₆ PUFAs decreased with time in the faeces of M. myotis (ANOVA, F₃,2₅ =6.01, p=0.003) and less pronouncedly in the faeces of M. daubentonii (ANOVA, F₂,₁₅ =1.36, p=0.287), while a temporal decrease in concentrations of ω₃ PUFAs occurred in the faeces of M. mystacinus (ANOVA, F₃,₂₀ =6.26, p=0.004) (Fig. 3, Table 2). The temporal differences in DHA and LIN concentrations were not significant for any species, except for DHA in M. mystacinus.
(ANOVA, \(F_{3,20}=4.16, p=0.019\)) (Fig. 3, Table 2). When we compared the samples collected during the same days (mid-May), total \(\omega 3\), total \(\omega 6\) and \(\omega 3/\omega 6\) values were different (Tukey’s HSD, \(p<0.005\)) between all pairs of species, except \(\omega 6\) PUFAs between \(M. mystacinus\) and \(M. daubentonii\) (ANOVA, \(F_{2,21}=16.01, p=0.677\)) and the \(\omega 3/\omega 6\) ratio between \(M. mystacinus\) and the other two species (Table 4). The ratio DHA/LIN was different between the species (ANOVA, \(p<0.05\)) except for \(M. mystacinus\) vs. \(myotis\) (ANOVA, \(F_{2,21}=5.44, p=0.633\)). The DHA and LIN concentrations did not differ between the species (Kruskal-Wallis, \(p>0.146\)) (Table 4). The GLMs for the PUFAs indicated that the variation of \(\omega 3\) PUFAs was explained mainly by species (\(p<0.001\)) with date not significant (\(p=0.107\)), while for \(\omega 6\) PUFAs, both species (except \(M. mystacinus\)) and date were significant (\(p<0.001\)) (Table 3).

**Fig. 4** Mean values ± standard errors of A. \(\omega 3\) PUFAs, B. \(\omega 6\) PUFAs and, C. DHA/LIN ratio from the faeces of *Myotis myotis* (n=29), *M. mystacinus* (n=24) and *M. daubentonii* (n=18). %TFA= % of total fatty acid. The groups with different letter were different.

**Table 3.** Results from the general linear models (GLMs) applied to each stable isotope (\(\delta^{15}N, \delta^{13}C, \delta^{34}S\)) and fatty acid parameter (\(\omega 3, \omega 6\) PUFAs) with species (*Myotis myotis, M. daubentonii, M. mystacinus*) and date as explaining variables.

<table>
<thead>
<tr>
<th>parameter</th>
<th><em>M. myotis</em></th>
<th><em>M. daubentonii</em></th>
<th><em>M. mystacinus</em></th>
<th>date</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\delta^{15}N)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\delta^{13}C)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.033</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\delta^{34}S)</td>
<td>&lt;0.001</td>
<td>0.225</td>
<td>0.002</td>
<td>0.094</td>
</tr>
<tr>
<td>(\omega 3) PUFAs</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.107</td>
</tr>
<tr>
<td>(\omega 6) PUFAs</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.858</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The statistically significant values are indicated in bold.
**Linear discriminant function analysis**

The linear discriminant analysis based on stable isotope values successfully assigned all faecal samples of *M. myotis* and *M. daubentonii* to the correct species (100%; Table 5). In 87% of the cases, *M. mystacinus* samples were attributed correctly. The rest were misclassified as *M. myotis* or *M. daubentonii*. The linear discriminant analysis based on fatty acids (ω3, ω6 and ω3/ω6) performed less well, yet was still able to classify faeces to the correct species, 97% of *M. myotis* faeces and 83% of *M. daubentonii* and *M. mystacinus* faeces were assigned correctly (Table 5).

**DISCUSSION**

Both tracers, stable isotope and fatty acid signatures, successfully discriminated among the three study species in terms of the aquatic and terrestrial origins of their diet, consistent with known feeding preferences. The fact that there was no difference in stable isotope and fatty acid signatures between the faecal samples from the lake-near and the lake-far roosts of *M. myotis* confirms that individuals of this species strictly rely on terrestrial prey irrespective of proximity to aquatic ecosystems.

**Stable isotopes**

Faecal stable isotope signatures showed that the three species occupy different isotopic niches consistent with our predictions: *M. myotis* feeds on terrestrial prey and had higher δ¹³C and lower δ¹⁵N values than *M. daubentonii*, an ecological specialist who forages over water bodies where catches insects as they emerge. The indication of an aquatic, a terrestrial or a mixed diet signature was more distinct based on δ¹⁵N than on δ¹³C or δ³⁴S values. The more a species depended on aquatic food, the higher δ¹⁵N values (δ¹⁵N: *M. myotis* < *M. mystacinus* < *M. daubentonii*). It is also possible that higher δ¹⁵N values imply feeding on prey from higher trophic levels. Although we cannot exclude piscivory by *M. daubentonii* as it has been shown that it is able to catch small fish (Siemers et al. 2001), this is rather unlikely since there are no studies reporting fish remnants in the faeces of this species.

The intermediate values of δ¹⁵N, the concentrations of ω3 and ω6 PUFAs, the concentration of DHA and the ω3/ω6 and DHA/LIN ratios recorded in the faeces of *M. mystacinus* were indicative of the mixed diet of this species. This would not have been revealed as clearly by using a single stable isotope (δ¹³C, δ³⁴S). The triple approach (δ¹³C, δ¹⁵N and deuterium) was also successful in identifying breeding origins of migrating bats (Popa-Lisseanu et al. 2012). The higher variability in the isotope signatures in the values of *M. daubentonii* and *M. mystacinus* compared to *M. myotis* (Fig. 2) is likely due to a higher variability in the diet or/and in the feeding
habitat (Abdennadher et al. 2011). However, since the species are feeding in different systems a direct comparison of diet breadth was not possible as the dietary baseline signatures are different.

Our study includes the calculation of δ34S signatures, rarely used in mammalian ecology. Signatures of δ34S can provide paleo-dietary information for mammals (Richards et al. 2003), detect sulphur polluted diets (Peterson & Fry 1987) or be applied when the species feed near the sea or prey from water with different salinities, as the δ34S signature is related to salinity (Fry & Chumchal 2011). Since δ34S can refine information obtained by δ15N and δ13C, our data support the recommendation that it should be routinely used (Privat 2004). Our results suggest a difference in δ34S values between systems, with higher δ34S for bats that rely on aquatic prey than on terrestrial, which provides additional evidence for differences in aquatic versus terrestrial organic material.

**Fatty acids**

The faecal PUFA profiles, similar to our stable isotope data, indicate that *M. myotis* and *M. daubentonii* occupy different niches, while *M. mystacinus* had some overlap, in terms of aquatic and terrestrial origin of prey (Fig. 5). The PUFA profiles reflected, as expected, feeding preferences for terrestrial and/or aquatic prey. Faeces of *M. myotis* had higher concentrations of linoleic acid (LIN) and total ω6 PUFAs than *M. daubentonii*, in line with our expectations. Faeces of *M. daubentonii*, known to eat aquatic prey, had significantly higher concentrations of ω3 PUFAs. The trends for higher ω3 PUFAs indicating a more aquatic diet and higher ω6 PUFAs indicating a more terrestrial diet agree with other studies on terrestrial and aquatic animals (Napolitano 1999; Koussoroplis et al. 2008; Fontaneto et al. 2011).

The ω3/ω6 and DHA/LIN ratios decreased with increasing terrestrial prey the species is assumed to consume. Similar tendencies have been reported for stream food webs (macroinvertebrates, allochthonous and autochthonous matter) (Torres-Ruiz et al. 2007) and semi-aquatic mammals (Koussoroplis et al. 2008). While *M. myotis* had low ω3/ω6 and DHA/LIN ratios due to its terrestrial diet, *M. daubentonii* had the highest ratios, and *M. mystacinus* had intermediate values in ω3/ω6 and DHA/LIN ratios and thus obviously relied on a mixed diet. Presumably, *M. mystacinus* consumed more aquatic than terrestrial prey, as its PUFA profile was closer to that of *M. daubentonii*. This proximity was also evident in the stable isotope values.
Fig. 5 Total ω6 PUFAs plotted against total ω3 PUFAs for Myotis myotis (n=29), M. mystacinus (n=24) and M. daubentonii (n=18), TFA= % of total fatty acid. Erm: Ermatingen, Lip: Lipperswil.

Temporal variation
The temporal variation in the tracers may be related to temporal differences in prey availability. During the study period the average air temperature gradually increased (from 14.7°C to 24.7°C) likely resulting in changes in relative abundance of certain prey species or the general composition of the insect community the bats fed on (e.g., Smukalla & Meyer 1988; Wickramasinghe et al. 2004). Also the stable isotope and fatty acid signatures of the insects may have changed seasonally (e.g., Grey et al. 2004; Torres-Ruiz et al. 2007). We followed a non-invasive approach, so did not catch bats to obtain faeces from individuals of known age or sex. This might have contributed to the differences in diet as for example the energy demand of female bats is increased during pregnancy and lactation period (Encarnação & Dietz 2006) and our samples probably included faeces from bats in different reproductive stages. Differences in hair stable isotopes (δ13C, δ15N, δ34S, hydrogen) do occur between males and females, and juvenile and adult individuals of the insectivorous bat, Eptesicus fuscus (Cryan et al. 2012).

Almost all parameters (δ13C, δ15N, δ34S, ω3 and ω6 PUFAs) differed between the three species, no matter whether we compared only the samples collected in the same period or all the samples. This implies that the stable isotope and fatty acid signatures of aquatic and terrestrial prey, regardless of the temporal variation we found, are different. It also indicates that these bats have stable preferences for aquatic or terrestrial prey, consistent with the conclusions from studies employing identification of prey remains in faeces.
**Stable isotope vs. fatty acid analysis. Applications in ecology**

Our results indicate a complementarity between the two tracers. The linear discriminant analysis assigned faeces to the correct species with similar success for both stable isotope and fatty acid signatures. Although absolute stable isotope values can differ between regions, dissimilarities also occur between different habitats within one region. The spatial scale at which stable isotope analyses remain comparable may differ, depending on the heterogeneity of the habitat. Thus, caution must be taken when comparing isotopic values of different regions and the baseline isotopic signatures must be known (Cabana & Rasmussen 1996).

While stable isotope signatures depend on the region, differences in fatty acids between aquatic and terrestrial systems are more universal. Thus, when baseline isotopic signatures are unknown for the study area, fatty acid signatures may be the preferred tracers. Stable isotopes, however, are superior when information for the individual level is required or when there is low availability of samples, as stable isotope analysis can be conducted even on a single faecal pellet. Fatty acid analysis, typically requires larger quantities of sample, which means that one sample has to be comprised of more than one pellet. If these are collected from a roost, fatty acid signatures reflect an integrated signature.

Since faeces (Barclay et al. 1991) and their stable isotopes (Salvarina et al. 2013) both provide information about the most recent food, they can be used to track short-term diet or habitat shifts and flexibility in feeding behaviour. Isotope signatures do not allow identification of prey items to the species level, as molecular or taxonomic analysis of the fragments would do, but they do allow tracking the type of feeding habitats. Investigating the aquatic versus terrestrial feeding preferences of the species could be used, for instance, for the conservation of endangered species, especially in areas with limited water resources. By examining the contribution of aquatic food in the diet of terrestrial predators, it is possible to determine which species depend on aquatic prey and predict possible consequences for these species in case of aquatic habitats eliminations and degradations.

Further questions that need to be addressed include the potential importance of certain fatty acids originating from different habitats in determining food quality for mammals and whether or not the availability of these fatty acids is associated with fitness consequences. In mammals, dietary deficiencies in $\omega_3$ PUFAs have been related to behavioural disorders (Brenna 2011).

In conclusion, we show that fatty acids (i.e., total $\omega_3$ PUFAs, $\omega_6$ PUFAs or single fatty acids such as DHA and LIN or their ratios) and stable isotopes (i.e., $\delta^{13}$C, $\delta^{15}$N, $\delta^{34}$S) in faeces can be used as ecological tracers for aquatic and terrestrial diet preferences of bats and potentially other mammals. Faeces can be used as an alternative to animal tissue when investigating recent diet and represent a non-invasive approach.
ACKNOWLEDGEMENTS

We thank Birgit Beck and Petra Merkel for help with stable isotope and fatty acid analyses, respectively, Dietmar Straile for helpful comments and, Jody Rintoul and Paul Preston for improving our English. Klaus Heck, Wolf-Dieter Burkhard, Katharina Greiner-Perth and Marcel Hasenmaier helped collect samples. We are also grateful to the editor and the two anonymous reviewers for comments that improved the manuscript. I.S. is a member of the International Max Planck Research School for Organismal Biology (IMPRS), Germany.

**Table 4.** Results of the ANOVAs and post-hoc tests and Kruskal-Wallis tests for the comparison of the fatty acid values between the species, using i) all the samples and ii) the samples collected in the middle of May only.

<table>
<thead>
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<th></th>
<th>all samples</th>
<th>samples only from mid May</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ω3</td>
<td>ω6</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>statistics</td>
<td>df=2,</td>
<td>df=2,</td>
</tr>
<tr>
<td>species with difference</td>
<td>X²=54.03</td>
<td>X²=37.38</td>
</tr>
<tr>
<td>test</td>
<td>Kruskal-Wallis</td>
<td>Kruskal-Wallis</td>
</tr>
<tr>
<td></td>
<td>myo-dau/</td>
<td>myo-mys</td>
</tr>
<tr>
<td></td>
<td>myo-dau/</td>
<td>myo-mys</td>
</tr>
<tr>
<td></td>
<td>all</td>
<td>all</td>
</tr>
</tbody>
</table>

|                         | ω3     | ω6     | ω3/ω6  | DHA   | LIN    | DHA/LIN |
| p value                 | <0.001 | <0.001 | <0.001 | 0.146 | 0.292  | 0.013   |
| statistics              | F₂,2₁=49.54 | F₂,2₁=16.01 | F₂,2₁=23.43 | df=2,  | df=2,  | F₂,2₁=5.44 |
| species with difference | all    | myo-dau/ | myo-dau/ | none  | none  | myo-dau/ |
|                         | myo-mys | myo-mys | myo-dau/ | none  | none  | mys-dau  |
| test                    | ANOVA  | ANOVA  | ANOVA  | Kruskal-Wallis | Kruskal-Wallis | ANOVA  |

myo: *Myotis myotis*, mys: *M. mystacinus*, dau: *M. daubentonii*. The statistically significant values are indicated in bold.
Table 5. Results of the linear discriminant function analysis for the stable isotope ($\delta^{15}$N, $\delta^{13}$C, $\delta^{34}$S) and fatty acid data (total $\omega$3 and $\omega$6 PUFAs and $\omega$3/$\omega$6 ratio).

<table>
<thead>
<tr>
<th>Species</th>
<th>Predicted classification</th>
<th>Prediction success</th>
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<tbody>
<tr>
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<td>Prior classification</td>
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<tr>
<td></td>
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</tr>
<tr>
<td>with stable isotopes</td>
<td>$M. myotis$</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>$M. mystacinus$</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>$M. daubentonii$</td>
<td>0</td>
</tr>
<tr>
<td>with fatty acids</td>
<td>$M. myotis$</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>$M. mystacinus$</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>$M. daubentonii$</td>
<td>0</td>
</tr>
</tbody>
</table>
CHAPTER IV

Seasonal bat activity related to insect emergence at three temperate lakes

Ioanna Salvarina, Dorian Gravier, Karl-Otto Rothhaupt

Manuscript in review in Journal of Mammalogy

ABSTRACT

Knowledge of aquatic food resources entering terrestrial systems is important for food web studies and conservation planning, especially when water availability in an area is limited. Bats, among other terrestrial consumers, often profit from aquatic insect emergence and their activity might be closely related to such events. To understand this relationship and search for seasonal patterns, we assessed bat activity using acoustic monitoring and caught emerging and aerial flying insects at three different lakes in three seasons. We predicted that insect availability and seasonality explain the variation in bat activity, independent of the lakes.

In all lakes, Chironomidae constituted the highest number of emerged insects. Seasonal patterns of emergence were uni- or bimodal with peaks at different times of the year (beginning of summer, end of summer, beginning of autumn) in each lake. Bat activity also showed seasonal fluctuations, but this did not necessarily follow insect emergence, probably because other factors, such as season, habitat characteristics, or bats’ energy requirements, play an important role as well. Bats were active throughout the night with some activity peaks, and the pattern of their activity also differed among lakes and seasons.

Lakes, regardless of their size or their characteristics, are important habitats for bats, as they support diverse bat communities and activity throughout the night and the year, when bats are active. Our study highlights that there are seasonal and spatial differences in insect emergence, bat activity and its hourly nocturnal pattern, that should be considered when investigating aquatic-terrestrial interactions or designing conservation or monitoring plans for bats.
INTRODUCTION

Aquatic insects can enter the terrestrial landscape and become a part of its food web. Bats and other terrestrial animals often feed on aquatic insects, thus water bodies and riparian areas are important habitats for bats (e.g., Walsh & Harris 1996; Grindal et al. 1999), and thus are also important areas of conservation concern. Additionally, water availability has been positively related with bat species richness (McCain 2007), therefore understanding the dependency of bats on aquatic food resources is crucial.

In Europe, all bat species are insectivorous and many of them include aquatic insects in their diets (Vaughan 1997). The evidence for this has been mainly from identification of prey remains in faeces (reviewed by Vaughan 1997), and to a lesser degree from stable isotopes (Lam et al. 2013), molecular analyses on faeces (e.g., Krüger et al. 2013), and experiments (Fukui et al. 2006). However, the amount of aquatic insects entering the terrestrial systems that are available to terrestrial consumers and whether this food resource fluctuates seasonally is not well known, particularly with responses in bat activity. The importance of aquatic insects as a food resource for bats may differ seasonally, for example in early spring when other prey availability is low (Fukui et al. 2006).

Aquatic resources in many areas of the world are limited or degrading. Numerous water bodies and bat species are under conservation. To set priorities in conservation policies for bats and aquatic systems and to understand better bats’ dependence on aquatic systems it is also important to know how much bats use aquatic resources. Greig et al. (2012) performed a mesocosm experiment that showed that water warming has significant effects on the magnitude, composition and phenology of insect emergence. Studying aquatic-terrestrial interactions is an important topic in ecology with increasing interest (e.g., Gratton et al. 2008; Bartrons et al. 2013) and implications, such as in helping to: a) investigate and possibly predict the effects of climate change and eutrophication of waters on terrestrial consumers, b) study food webs, c) manage the conservation of ecosystems and species effectively, and, d) track transfer of contaminants from aquatic to terrestrial systems (Mogren et al. 2013).

Our aim was to search for general patterns in bat activity at the shores of three different lakes, in the same area, with respect to season and insect abundance. We collected emerged aquatic insects during the spring, summer, and autumn. We simultaneously monitored bat activity at the shores of these lakes, using acoustic monitoring, which is an effective and non-invasive method for bats.

Eighteen bat species have been reported in the area where the lakes are located (Hinweise LUBW 2013, Fledermausschutz Thurgau 2014, Table 1, Supplement). All are insectivorous with
varying degrees of specialization on aquatic or terrestrial insects (Table 1, Supplement), and often with different foraging strategies and habitat preferences. We expected insect availability to partially explain bat activity, and predicted a seasonal effect. Studies often acoustically sample bat activity for a limited number of hours in the night after the sunset, potentially ignoring important activity later in the night or before sunset. Thus, another objective of our study was to explore the nocturnal (throughout the night) bat activity patterns seasonally and spatially. Emergence behaviour of bats is suggested as a monitoring tool of animal responses to long-term changes in climate, as it is related to climate and weather conditions (Frick et al. 2012).

MATERIALS AND METHODS

Study sites
The study was conducted at three lakes in South Germany that has a temperate seasonal climate (Fig. 1). Lake Constance is a deep (max. depth 254 m), large (500 km²), pre-alpine, oligotrophic (low in nutrient content) lake, situated in between 3 countries (Germany, Switzerland, Austria). The sampling location (47°41'27.72"N, 9°12'08.18"E) was near the city of Konstanz, in Upper Lake Constance, which is less than 10 m deep and is considered as a shallow area (littoral zone) (Baumgartner et al. 2008). The shore, near our study area, was composed of forest’s patches, meadows, small pastures, gardens, and orchard. Mindelsee (47°45'06.95"N, 9°01'24.80"E) is a shallower (max. depth 12 m), smaller, mesotrophic to eutrophic lake, included in a nature reserve. We sampled in the southern, steeper littoral zone which is bordered by a hill forested mainly with beech trees (Smukalla & Meyer 1988). Siechenweiher (47°41’47.33”N, 9°16’.54.09”E) is a shallow (max. depth 2.5 m), highly eutrophic (Seenprogramm 2010), small (about 0.05 km²) fishing pond at the edge of the town of Meersburg. It is situated between a residential area and a busy road, however its watershed (227 ha) is composed of forests (10%) and agricultural land (75% of which 22% is meadows, 35% arable land and 43% orchard).

We conducted fieldwork during 2 years (from July till October 2011 and from April till June 2012, plus 2 samplings in May and June 2011) to have data covering one ‘bat year’ (spring, summer, autumn), when bats are active.

Insects
Emerging aquatic insects were collected with floating traps (surface: 2500 cm²: 50x50 cm) that looked like pyramids with a bottle of killing solution (either alcohol 80% or 1 alcohol: 1 ethylene glycol: 1 tap water) on the top. The traps were constructed at the University of Konstanz using a model of similar traps used in other studies (e.g., Hagen & Sabo 2012).
Fig. 1 Map of a. Europe where the study area is indicated with an arrow and, b. all the study lakes (left) and the sampling location (right) in each lake: Lake Constance, Mindelsee and Siechenweiher.

Three to five traps were placed in Lake Constance (at about 1, 2, 3, 6, 8 m- the water level varied about 175 cm), and three traps in the other lakes (at 1, 2, 5m in Mindelsee and at 1-1.5 and 2 m in Siechenweiher). The traps remained on the water from May to October 2011 and from April to June 2012. We sampled insects every 5 days, with some variation due to logistic issues (e.g., bad weather). We also collected separate insect samples the nights (from sunset till sunrise) that we recorded the bat activity. To have an idea of the insects that had emerged during the day and previous nights we used the sample of emerged insects during the previous days and nights as due to logistic reasons it was difficult to make one-day collections. We calculated insect emergence per hour and per trap. Hereafter, we will refer to the emerged insects caught during the 5 days and nights as ‘day emerged insects’ (hour⁻¹trap⁻¹) and to the insects emerged during bat recording nights (hour⁻¹trap⁻¹) as ‘night emerged insects’.

Aerial flying insects were caught using one Malaise trap constructed at the Limnological Institute, University of Konstanz (built with model the trap from Bioform®; 295x175x94 cm). The Malaise trap, due to logistical constrains, was set up only 3 hours before the sunset, to give a picture of insects flying during the day. The first insect sample was collected at sunset. The
emergence rate per hour that corresponds to these 3 hours will be referred as ‘day aerial insects’. The sample from sunset till sunrise per hour corresponded to the ‘night aerial insects’. The Malaise trap was randomly orientated to avoid bias due to wind and a possible corridor of flying insects. The aerial flying insect collection was conducted only when bats were recorded during April-June 2012.

The insect samples were counted and classified in the laboratory to order or family level (based on: Roth 1974; Borror et al. 1989; Nilsson 1996, 1997). Usually, the Diptera were identified to family level (e.g., mainly Chironomidae, Simulidae). The body length (without antennas or other appendages) of the insects was measured and associated to a class length (mm): <3; 3-6; 6-9; >9 mm.

To calculate the dry biomass of the emerged insects we oven dried (at 50 °C for 48 hours to constant mass) and then weighed representative samples of the main taxa, Chironomidae, Simulidae and Trichoptera from each lake, season and size class, whenever possible. To get the dry biomass the median weights of Chironomidae from each size class, season and lake were then multiplied by the total number of the Chironomidae of that size class in each sample (Baumgärtner & Rothhaupt 2003). For the Trichoptera the median per lake was used and for Simuliidae the median from Lake Constance specimens was used. For the other Diptera and the remaining taxa we used the weight of size class 2 of Chironomidae of the respective lake and season each time.

The emergence rate was calculated as number of individuals trap⁻¹ hour⁻¹ and the emergence production as g trap⁻¹ hour⁻¹. Due to the different night length through the seasons we preferred to express the insect emergence per hour so that it corresponds to bat activity that was also expressed per hour. For the emerged insect emergence, the mean values from all samples/traps of the same day(s) were used for the data analysis, unless some samples were destroyed or lost.

Bats
Bat activity was assessed with acoustic monitoring during 3 nights (from about 20 min before the sunset till sunrise) per sampling month at each lake from July to October 2011 and April-June 2012 (plus two samplings in May and June 2011). We used an automatic bat recorder, Batcorder (Ecoobs, Nurnberg, Germany), hanging on a 2 m pole, placed about 3-4 m from lakeshore. Batcorder’s microphone is omnidirectional, thus it records activity over the lake and on the shore.

In all recordings we used the same mode (“Auto+Timer”) and the same settings of the batcorder (quality: 20; threshold: -27 dB; post-trigger: 400 ms; critical call frequency: 16 kHz, sample rate: 500 KHz). The distance in which a recorder can record bat calls varies according to species, individuals, habitats, weather conditions (humidity, air temperature) and the recorder’s
settings and sensitivity. Some species have loud calls, such as *Nyctalus* spp, *Eptesicus* spp. while others have low amplitude calls like *Myotis* spp. or *Plecotus* (though *Plecotus* may be as loud as *Nyctalus* sometimes). In between are *Pipistrellus* species. The batcorder, with the specific settings we used, can record *Pipistrellus* species up to 10 to 15 m, *Myotis/Plecotus* species up to 2 to 10 m and *nyctaloid* species (genera *Nyctalus, Vespertilio, Eptesicus, Tadarida*) up to 20 to 40 m (pers. comm. Volker Runkel, Ecoobs).

Usually bats stop hunting when it rains (Mcaney & Fairley 1988; Roué & Barataud 1999) and researchers usually record bats when there is no rain, low wind (<10 km/h), and at least a medium temperature at the sunset (e.g., at least 10 °C, Kusch & Idelberger 2005). We followed these recommendations as much as possible. Bat activity was defined as seconds of recording of bat passes per hour of recording in each night. In each recorded sequence of calls there are included a pre- and post-trigger time of 50 ms and the intervals between the calls. If there was rain during the night the recording was stopped and that time was excluded from the total recording time. We also recorded the wind on a subjective scale from 0 (no wind) to 5 (strong wind) at the time of the sunset till midnight.

**Acoustic analysis**

For acoustic analysis we used software (from Ecoobs) that is specific for recordings made with batcorder: bcAdmin for the management of recorded sessions and sequences; bcDiscriminator that recognizes and takes measurements on bat calls in each sequence; and batIdent that uses those measurements to give a potential identification (on a species or group level) with a probability of this identification to be correct. Since the above-mentioned programs do not permit listening to the recordings, the sequences that needed to be manually checked were exported to wav files and opened with Raven Pro (Bioacoustics Research Program 2011). All sequences identified only as ‘Chiroptera’ or ‘nothing’ were checked in Raven Pro. Most could be identified to the species, genus or group level, few remained as Chiroptera and those that were noise were deleted. The identification was done by only the authors (IS) to avoid bias. For the identification books (Tupinier 1997; Barataud 2002; Koordinationsstellen für Fledermausschutz in Bayern 2009), and papers (Russo & Jones 2002; Obrist et al. 2004) were used. We classified all the calls identified automatically with a probability of ≤70% in the previous lower identification level. The same was true for species, such as *M. alcatheo*, whose presence in the area is unlikely and has not been confirmed before (pers. comm. Wolfgang Fiedler).

We grouped *Pipistrellus nathusii* and *P. kuhlii*, together, even if they were identified automatically, as due to their similarities in call characteristics, it is very difficult to distinguish them only from echolocation calls. Nyctaloid species and *Myotis* species were also grouped
respectively for further analysis, due to their similarities in call characteristics and usually the low probability that BatIdent identifies them.

Feeding buzzes are sequences where the pulses decrease gradually in duration and are also characterized by short interpulse intervals (Griffin 2001). They are produced when a bat is hunting an insect. A number of sequences (3249 sequences, 25\% of the total number), randomly selected, covering all recording sessions were checked manually (visually and acoustically), in Raven Pro, for feeding buzzes.

**Statistical analysis**

We searched with linear regression for correlations between day emerged insects (from 5 days and nights preceding the recording night) and night emerged insects; between insects and air temperature at the sunset; between total bat activity and activity of each species/group (P. pipistrellus, P. pygmaeus, P. nathusii/kuhlii, Myotis spp., M. daubentonii and nytaloids) with insect samples (number and biomass of day and night emerged insects, day and night aerial insects), and between total number of calls and calls with feeding buzzes. The total and species/group activity was compared among seasons (per lake) and among lakes (all seasons together and per season) using the non-parametric test Kruskal-Wallis. To test for differences between day and night aerial insects, we used the Mann Whitney Wilcoxon test. To investigate which factors explain bat activity in each lake we applied several general linear models (glm's), with the following explaining factors combined in various ways: total number of insects (separately: emerged day insects, emerged night insects, aerial flying insects), Julian day, air temperature at the time of sunset and wind. A model with lake as a random factor was, also, applied. All analyses were performed using the statistics package R version 3.0.3 (R Core 2014) run within R Studio interface R version 0.98.932 (R Studio 2013). Additional packages that were used were: pgirmess (Giraudoux 2014) for Kruskal-Wallis test, gridExtra (Auguie 2012) and ggplot2 (Wickham 2009) for plots and maps, and ggmap (Kahle & Wickham 2013) for maps.

**RESULTS**

**Insects**

The family Chironomidae accounted for the vast majority (82.5\%) of the aquatic insects caught in the emergence traps. The night emerged insects were positively related to the day emerged insects at Lake Constance ($R^2= 0.771, F= 27.05, df=8, p<0.001$), and Mindelsee ($R^2= 0.817, F=22.25, df=5, p=0.005$). The number of day and night emerged insects had a positive correlation with the biomass of day and night emerged insects, respectively ($R^2= 0.511, F=61.75, df=59, p<0.001$, and
R²=0.561, p<0.001, F=58.88, df=46, respectively). Therefore, in further analysis we used only insect numbers. The night emerged insects were positive correlated with the water temperature in all lakes (both ln(x+0.1) transformed, R²=0.43, p<0.001). More details on insect data and correlations with the water parameters are presented in Chapter V. Insect emergence showed seasonal fluctuations with a peak in August in Lake Constance and Mindelsee and a bimodal pattern with one peak in June and one in September in Siechenweiher (Fig. 2). The seasonal fluctuations were determined mostly by fluctuations in the Chironomidae.

The night emerged insects were positive correlated with the water temperature in all lakes (both ln(x+0.1) transformed, R²=0.43, p<0.001). More details on insect data and correlations with the water parameters are presented in Chapter V. Insect emergence showed seasonal fluctuations with a peak in August in Lake Constance and Mindelsee and a bimodal pattern with one peak in June and one in September in Siechenweiher (Fig. 2). The seasonal fluctuations were determined mostly by fluctuations in the Chironomidae.

The most abundant groups of aerial flying insects in all three lakes were Coleoptera (31% of total) and Chironomidae (16%). Terrestrial origin was attributed to 43% of all aerial flying insects, aquatic (mainly Chironomidae) to 17%, and the rest was not attributed to aquatic or terrestrial origin. The day aerial insects (caught during the 3 hours before the sunset) were significantly more than the night aerial insects (from sunset to sunrise) (p<0.021 in all lakes).

**Bats**

We recorded 13 bat species with similar numbers of species at each location (Table 1, Supplement), during 63 nights of recording. A higher number of *Myotis* species were recorded at Mindelsee; *Vespertilio murinus* was recorded only at Lake Constance and *Pl. auritus* only at Siechenweiher. The species number varied seasonally, with the highest number (12) being recorded in summer and the lowest (7) in autumn. Pipistrelloids accounted for most of the activity (92.3%) in all lakes and seasons. Nyctaloids and *Myotis* spp. contributed very little to the total activity (2.4% and 0.9% respectively). Mean activity of all bats and of pipistrelloids was highest in Siechenweiher, of *Myotis* spp. in Mindelsee and of nyctaloid in Lake Constance. The species with the highest activity from all lakes during the entire study were *P. pipistrellus* (29.7%), *P. nathusii/kuhlii* (51.3%), and *P. pygmaeus* (2.5%).

A small percentage (4.2%) of the total number of sequences that were checked included feeding buzzes. The number of sequences with feeding buzzes was positively related to the total number of calls checked (R²=0.402, F=37.02, p<0.001), so all the further analysis was done using all the calls.

The bat activity showed high seasonal fluctuations (Fig. 2) for the total and all species/group except *P. pygmaeus* and nyctaloids (p>0.1). A bimodal pattern of activity was noted in Lake Constance and Siechenweiher with a peak in late spring and early summer respectively and a smaller peak in autumn (Fig. 2). In Mindelsee the pattern seemed less clear and rather unimodal (Fig. 2). Differences in activity were noted among lakes and seasons (Fig. 3). The activity of *Myotis* spp. was significantly higher in summer compared to autumn in Lake Constance (0.030).
Variations in bat activity among lakes were more pronounced in summer (Fig. 3), when total activity and *P. pipistrellus* activity were significantly higher in Siechenweiher compared to Lake Constance (p<0.018); *P. pygmaeus* activity was higher in lakes Mindelsee and Constance compared to Siechenweiher (p=0.002); *P. nathusii/kuhlii* activity was higher in Mindelsee compared to the other two lakes (p=0.002), and *Myotis* spp. activity was higher in Mindelsee compared to Siechenweiher (p=0.042). In spring, only *P. pygmaeus* activity was higher in Mindelsee compared to Siechenweiher (p=0.013) (Fig. 3), while in autumn there were no significant differences in the bat activity among lakes.

The bat activity pattern throughout the night also varied both among the lakes and seasons (Fig. 4). In Lake Constance, in spring and summer the highest activity was recorded about 1 hour after sunset with a smaller peak later in the night before sunrise, while in autumn the activity was more evenly distributed throughout the night. In Mindelsee the peak of activity was in the second part of the night for all seasons, before sunrise, although there was a considerable activity throughout the night. In Siechenweiher the activity seemed to be also spread through the night, especially in spring.

**Fig. 2** Total bat activity (seconds/hour of recording) seasonally per lake and night emerged insects hour\(^{-1}\) trap\(^{-1}\) (night insects) and aerial flying insects (hour\(^{-1}\) trap\(^{-1}\)). Note the different scales in the
plots for bat activity and that the traps used for emerged aquatic and aerial flying insects are different, sample different areas and thus are not comparable. Smoothing was done (gam method) for total bat activity and emerged aquatic insects. One outlier for bat activity from Siechenweiher was excluded.

**Bat activity-insects**

Bat activity had a positive correlation with the day and night emerged insects (p<0.03) (Fig. 5), and the aerial flying insects (p<0.004) when all lakes and seasons were considered together. However, this correlation was not always significant when the analysis was done per lake (e.g., at Lake Constance and Mindelsee) or per bat species/group (Table 1). Most of the significant correlations were weak (e.g., *P. pygmaeus* and day emerged insects, $R^2=0.118$, p=0.007) (Table 1) and there was no significant correlation between bat activity and insects noted in Mindelsee. Only in Siechenweiher seemed the bat activity to correlate with the aquatic insect pattern (Fig. 2). In spring, in Lake Constance and Siechenweiher the bat activity seemed to increase similarly with the aerial flying insect numbers (Fig. 2).

Fig. 3 Total bat activity and activity per species/group (seconds/hour of recording) per lake and per season. One extreme value from Siechenweiher was excluded as outlier. LG (red): Lake Constance, MI (green): Mindelsee, SI (blue): Siechenweiher.

The best general linear models per lake (according to AIC value and plots) were those with night insects, Julian day and wind. However, insects explained part of the total bat activity
significantly (p<0.007) only in Lake Constance and in Siechenweiher. Wind although included in the best models did not explain the bat activity. Other models that were interesting were those with Julian day instead of mean air temperature (at the time of the sunset), then only temperature explained part of the variation in bat activity in Mindelsee and Siechenweiher (p<0.02). When we included the night aerial flying insects in the models with the Julian day and the night emerged insects (for the spring and early summer in 2012), the aerial flying insects didn’t explain significantly the variation of the bat activity in none of the lakes. The model with lake as a random factor, and the night emerged insects and Julian day as fixed factors, showed that the insects per hour explained part of the variation of the bat activity (p=0.002).

Table 1: Statistical significant results (p>0.05) and R² of the linear regressions between bat activity (seconds of activity hour⁻¹ of recording) and insects (hour⁻¹, trap⁻¹) per lake. Notice that in Mindelsee there were no significant correlations found.

<table>
<thead>
<tr>
<th>Model</th>
<th>Lake Constance</th>
<th>Siechenweiher</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. daubentonii-day insects biomass</td>
<td></td>
<td>R²=0.270, p=0.033</td>
</tr>
<tr>
<td>Myotis spp-day insects biomass</td>
<td></td>
<td>R²=0.562, p=0.001</td>
</tr>
<tr>
<td>P. kuhli/nathusii-day emerged insects</td>
<td></td>
<td>R²=0.371, p=0.01</td>
</tr>
<tr>
<td>P. kuhli/nathusii-night emerged insects</td>
<td></td>
<td>R²=0.320, p=0.028</td>
</tr>
<tr>
<td>P. nathusii/kuhlii- day aerial insects</td>
<td></td>
<td>R²=0.896, p=0.004</td>
</tr>
<tr>
<td>P. nathusii/kuhlii- night aerial insects</td>
<td>R²=0.669, p=0.004</td>
<td></td>
</tr>
<tr>
<td>P. pipistrellus - day aerial insects</td>
<td></td>
<td>R²=0.845, p=0.009</td>
</tr>
<tr>
<td>P. pipistrellus-night aerial insects</td>
<td>R²=0.645, p=0.005</td>
<td></td>
</tr>
<tr>
<td>P. pipistrellus-day emerged insects</td>
<td></td>
<td>R²=0.319, p=0.018</td>
</tr>
<tr>
<td>P. pipistrellus-night emerged insects</td>
<td></td>
<td>R²=0.476, p=0.004</td>
</tr>
<tr>
<td>total bat activity-biomass day emerged insects</td>
<td>R²=0.271, p=0.032</td>
<td></td>
</tr>
<tr>
<td>total bat activity-biomass night emerged insects</td>
<td>R²=0.401, p=0.011</td>
<td></td>
</tr>
<tr>
<td>total bat activity-number day aerial insects</td>
<td>R²=0.886, p=0.005</td>
<td></td>
</tr>
<tr>
<td>total bat activity-number day emerged insects</td>
<td>R²=0.327, p=0.016</td>
<td></td>
</tr>
<tr>
<td>total bat activity-number night aerial insects</td>
<td>R²=0.749, p=0.001</td>
<td></td>
</tr>
<tr>
<td>total bat activity-number night emerged insects</td>
<td>R²=0.401, p=0.011</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Our results showed seasonal variability in bat activity and insect emergence at three temperate lakes, in South Germany. Insect emergence partially explained the variation in bat activity in Lake
Constance and Siechenweiher, but not in Mindelsee. In Siechenweiher, the bat activity seemed to follow fluctuation patterns of the aquatic insects. In the spring, at both Siechenweiher and Lake Constance, bat activity seemed to increase as the aerial insects increased.

We assessed bat activity using acoustic monitoring, an effective and non-invasive method (e.g., Lintott et al. 2013). Captures using nets accounted for 63.5%, and acoustic sampling for 86.9% of the combined species present in the study area (O’Farrell & Gannon 1999). We recorded most of the expected species in the region (Table 1, Supplement), however we do recognize that some species could have not been recorded due to their low calls or the height of their flight is too high (e.g., Pl. auritus). Almost the same number of species were recorded in all the lakes, however Mindelsee had the highest species richness, Myotis species diversity, and activity. This can be attributed to the presence of a nearby forest that might have provided more roosting possibilities (Kunz & Lumsden 2003). The drop in the number of species and activity in autumn could be related to low temperatures and low insect availability that might drive bats to enter torpor or migrate. Particularly the species Vespertilio murinus, N. noctula and P. nathusii are known to migrate. However, records of P. nathusii in the region of Konstanz were higher in early spring and autumn and N. noctula were also found during winter, thus a part of the population might overwinter there (e.g., Bastian 1988; Braun & Dieterlen 2003). There is also evidence for northeastern migration from Konstanz, especially of N. noctula females in spring (Dechmann et al. 2014).

The high activity of nyctaloids in Lake Constance compared to the other lakes could be due to their needs to forage in wider areas and the openness of the lake compared to the others. Pipistrelloids had, by far, the highest activity throughout all seasons and at all lakes. Not only are the most abundant species in the region (Braun & Dieterlen 2003) but they are also easier to record. P. pipistrellus and P. nathusii/kuhlii had higher activity in the Siechenweiher, probably due to its proximity to a town, as some Pipistrellus species are known to roost in human-made constructions (Sattler et al. 2007). However, P. pygmaeus had almost negligible activity in Siechenweiher.

Bat activity differences among the lakes could be related to differences in lake characteristics (e.g., water quality, trophic condition), or other factors, such as surrounding habitat, proximity to bat roosts and perches, commuting routes, microclimate, and wind exposure. Wind per se did not have a significant effect on bat activity, but we were avoiding recordings in harsh weather conditions. However we speculate that waves in Lake Constance, as this place is open and more affected by wind, might explain the low bat activity there. Bats avoid rough surfaces and wavy waters because they interfere with echolocation (Warren et al. 2000). Myotis species are known to avoid areas of rivers with rapids (Lundy & Montgomery 2010).
Lake size might also explain bat activity differences among the lakes. Although Siechenweiher had low insect emergence per square meter, the small size might have attracted bats from the surrounding area for drinking or feeding. In contrast, a large lake the size of Lake Constance, which had longer lakeshores, could have had lower bat density at the site of our recording location. Mindelsee, which was of intermediate size, had higher bat activity than Lake Constance, but this could have been due to the availability of habitats. Our results suggest the importance of the size of lakes, particularly small to medium-sized ones, which might be crucial habitats for terrestrial consumers, especially in an area where water resources are limited. This is in agreement with studies done in arid areas, where bat species richness and activity have been found to increase with pond size, and an experimental reduction of pond size led to a reduction in bat species richness and activity, and affected the bat community composition (Razgour et al. 2010).

Differences of bat activity and species presence were not only spatial, but also seasonal. Higher bat activity in early spring could reflect the high energetic demands of bats after hibernation. High activity in spring and summer could possibly be due to pregnancy, lactation, or spermatogenesis, which also increase energetic requirements of bats. Encarnação et al. (2010) found that differences in foraging activity of *M. daubentonii* were more influenced by energy demands than food availability. Male *M. daubentonii* had higher activity during mid-summer when spermatogenesis occurs (Encarnação et al. 2006).

Positive correlations between insects and bat activity were found for Siechenweiher and Lake Constance. Correlations between bat activity and aquatic insect emergence were weak in general, and absent in Mindelsee, probably because the recorded species might feed partly or not at all on aquatic insects. The stronger correlations that were found between bat activity and aerial flying insects, as in spring at Lake Constance, also imply that bats do not depend only on aquatic insects. The species known to feed almost exclusively on aquatic insects, *M. daubentonii* and *P. nathusii* unfortunately could not be discriminated, in most of the cases or all cases, from congeneric *Myotis* and *P. kuhlii* respectively, that feed on terrestrial diet. *Pipistrellus pipistrellus*, which showed highest activity, is considered a generalist, while *P. nathusii* is often associated with aquatic habitats (Vaughan et al. 1997). Both *P. pygmaeus* and *P. kuhlii* feed on both terrestrial and aquatic insects. Insect availability was a poor predictor of bat activity in previous studies as well (e.g., Wolbert et al. 2014). Especially in Mindelsee, no correlations were found for any of the species/group and type of insects, and so we conclude that other factors characteristic of this lake are explaining bat activity.

Our study can only indirectly show the relationship between the emergence of aquatic insects and bat activity. Detecting causal relationships requires a more experimental approach, such as by
Fukui et al. (2006) who manipulated emerging insect numbers from a river in Japan and showed the correlation between bat activity and aquatic insects. Especially in the spring bat foraging activity on emerging insects was higher in the control areas than in the treatment where emergence was prevented.

In a study in Sweden, bat activity was better explained than in our study (DeJong & Ahlé 1991). Possibly, aquatic insects are more important resources in cases when terrestrial prey is limited. In Germany, food for bats is almost always available, except during hibernation (Zahn et al. 2007) and probably early spring, however this might not be the case in Sweden. Other studies have found that bat activity was influenced by both habitat structure and insect availability (e.g., in riverine habitats: Hagen & Sabo 2011), or by air temperature more than invertebrates (O’Donnell 2000). Temperature determined if bats fly at all during one night, while invertebrate abundance determined how long they feed (O’Donnell 2000).

The correlations between bat activity and insects were weak probably also because total activity is not necessarily feeding activity. Echolocation calls were the majority of the recorded sequences, but we detected social calls and feeding buzzes. Total activity was positively correlated with feeding buzzes, which is true for similar studies (e.g., Rainho 2007). Thus, total activity is considered a good indication of foraging activity. Feeding buzzes show that bats are following insects, and while the result of the hunt might be unknown, we at least know that the bats were hunting.

The nocturnal pattern of bat activity differed between lakes, possibly reflecting differences in habitat, microclimate, and proximity of roosts. The Pipistrellus species accounted for most of the activity in all the lakes, and so we do not expect the species composition to be responsible for these activity differences. The nocturnal pattern of bat activity seemed to follow the usual bimodal peaks of insect emergence at dawn and dusk (e.g., Smukalla & Meyer 1988; Rydell et al. 1996) in the spring/summer for Lake Constance, and in the summer for Siechenweiher. The additional peak we found in the Siechenweiher, in the middle of the night, in the spring could be due to lactating bats that make more foraging trips than females at other reproductive stages (e.g., Lučan & Radil 2010; Rintoul & Brigham 2014). A possible explanation for the absence of a specific pattern in the autumn could be the low insect availability that might drive bats to search longer for food, or due to competition, individuals might fly at different hours and places (Swift & Racey 1983). Seasonal variations in nocturnal activity, foraging time per night and time of departure and return to the roost have been reported elsewhere as well (Encarnação et al. 2010). For example, P. nathusii has been reported to show bimodal activity patterns when hunting over wooded sites, while it had unimodal post-midnight activity when hunting in open areas to avoid predators (Ciechanowski et al. 2009).
By examining hourly nocturnal activity pattern per season, differences among nights could be masked. In our study area, bats were active throughout the entire night, which was consistent with other studies (e.g., O’Donnell 2000). If, in places like Mindelsee, monitoring is done only few hours after sunset, considerable amount of activity will be missed. Therefore, we recommend when nocturnal pattern of bats is unknown, to conduct a pilot study first with a few nights of full recordings in each season and then decide if only few hours of monitoring are enough and when these hours should be.

**Summary, conclusions and future directions**
Seasonal variation was found in bat activity and insect emergence in all the lakes. There was no general pattern of bat activity for the studied region, and activity peaks were not necessarily dependent on insect emergence as predicted. We suspect that other factors might have played a larger role in explaining the bat activity variations, such as habitat type, microclimate, size of the lake, and seasonal energy requirements of bats, especially in one of the lakes (Mindelsee). Bats were active throughout the entire night, but their hourly patterns varied seasonally and spatially.

We indirectly examined the effect of insect emergence to bat activity on a seasonal basis. Due to the limitations of our study (not considering habitat differences, one replicate of each lake and season), our results do not allow us to distinguish which factor was the most significant. However, our data show that, indeed, lakes are important for bats as they support diverse bat communities with varying seasonal activity. Field experiments that control insect availability might provide better insight into to what extent aquatic insect resources influence bat activity. Comparative studies in areas with limited water availability might yield insight into the flexibility and resilience of bat species to changing environmental conditions. We showed that lakes are important sources for insect biomass and for adjacent terrestrial systems but may not be straightforward predictors of the behavior of terrestrial consumers. Our findings may also be of use for the conservation of bat species and lakes.

**ACKNOWLEDGEMENTS**
We are grateful to Kamran Safi, Hans-Günther Bauer, and Elizabeth Yohannes for help in the design of the study and advices during it, and to Barbara Helm, Yann Gager and Teague O’Mara for providing useful feedback on the manuscript and to Akiko Matsuda and Paul Preston for the language corrections on the manuscript. We also thank Christian Fiek, Salman Said, and Markus Köpf for technical support and help in the field, and Martin Wolf, Alfred Sulger and Josef Halder for technical assistance. We are thankful to Klaus Heck, Wolfgang Fiedler, and Dina Dechmann for providing equipment and information concerning bats in the region and to Volker Runkel
(Ecoobs) for his support on the use of batcorder and bat analysis software and to Dietmar Straile for discussions about statistics. We also thank BUND and Kai-Steffen Frank, and the Angelsportverein Meersburg e.V. for allowing us to conduct fieldwork in Mindelsee and Siechenweiher respectively.

Fig. 4 Box-plots of the hourly total bat activity (seconds of activity per hour of recording) per lake and per season. Hourly intervals are calculated according to the sunset time. 0: sunset. Note the different scales of Y-axis that were kept for better clarity of the hourly pattern although they do not permit easy comparison of the activity. LG: Lake Constance, MI: Mindelsee, SI: Siechenweiher.
Fig. 5. Bat activity (seconds of activity/hour of recording) related to night insect emergence (number of insects/hour/trap) at all the lakes from: a) total bat activity, b) *P. pipistrellus*, c) *P. pygmaeus* and, d) *P. nathusii/kuhlii*. Both parameters are ln transformed.
SUPPLEMENT

Table 1
Bat species reported in the study area and their feeding habits (and references). The study lakes where some species were recorded in the current study are mentioned. Lakes: CO: Constance, MI: Mindelsee, SI: Siechenweiher

<table>
<thead>
<tr>
<th>Species</th>
<th>Diet</th>
<th>References for diet information</th>
<th>present study</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myotis bechsteinii</td>
<td>Lepidoptera, Aranea, Brachycera, Neuroptera, Diptera, Coleoptera, Orthoptera</td>
<td>Vaughan 1997; Safi &amp; Kerth 2004</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>Myotis blythii</td>
<td>bush crickets, cockhafers, lepidopteran larvae, Orthoptera</td>
<td>Arlettaz 1996; Safi &amp; Kerth 2004</td>
<td></td>
<td>found in nearby Switzerland</td>
</tr>
<tr>
<td>Myotis brandtii/mystacinus</td>
<td>Diptera, Chironomidae, Simulidae, Tipulidae, Anisopodidae, Culicidae, Arachnida, Lepidoptera, Coleoptera</td>
<td>Vaughan 1997; Safi &amp; Kerth 2004</td>
<td>√</td>
<td>M. brandtii is reported in the broad region. M. mystacinus is most probable in the study area</td>
</tr>
<tr>
<td>Myotis daubentonii</td>
<td>Ceratopogonidae, other Diptera, Trichoptera, Ephemeroptera, Neuroptera, aquatic Diptera, mostly Chironomidae</td>
<td>Vaughan 1997; Swift &amp; Racey 1983; Flavin et al. 2001; Safi &amp; Kerth 2004; Todd &amp; Waters 2007</td>
<td>√</td>
<td>aerial hawking above water surface and trawling from water surface</td>
</tr>
</tbody>
</table>

Continued in the next page
### Table 1 Suppl. Continued from the previous page

<table>
<thead>
<tr>
<th>Species</th>
<th>Insects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Myotis myotis</em></td>
<td>Coleoptera (Carabidae: carabid beetles), Lepidoptera, Orthoptera (Gryllotalpidae: mole crickets)</td>
<td>Arlettaz 1996; Safi &amp; Kerth 2004 √ √</td>
</tr>
<tr>
<td><em>Nyctalus noctula</em></td>
<td>Lepidoptera, Diptera, Coleoptera, Tipulidae, Culicidae, Trichoptera, Chironomidae etc</td>
<td>Gloor et al. 1995; Vaughan 1997; Kanuch et al. 2005 √ √ √</td>
</tr>
<tr>
<td><em>Pipistrellus kuhli</em></td>
<td>Culicidae, Lepidoptera, Chironomidae, Ceratopogonidae, Hymenoptera, Brachycera, Tipulidae, Coleoptera</td>
<td>Goiti et al. 2003; Safi &amp; Kerth 2004</td>
</tr>
<tr>
<td><em>Pipistrellus nathusii</em></td>
<td>mainly Diptera, Lepidoptera but also Ephemeroptera, Trichoptera, Coleoptera etc, mainly aquatic Diptera Chironomidae</td>
<td>Vaughan 1997; Safi &amp; Kerth 2004; Krüger et al. 2013 √ √ √</td>
</tr>
<tr>
<td><em>Pipistrellus pipistrellus</em></td>
<td>mostly Diptera (flies), Trichoptera, Ephemeroptera, Neuroptera, less Coleoptera, Lepidoptera, Diptera, Chironomidae, Ceratopogonidae, Tipulidae, Psychodidae, Anisopodidae, Empididae</td>
<td>Vaughan 1997; Swift et al. 1985 √ √ √</td>
</tr>
</tbody>
</table>

*Continued in the next page*
### Table 1 Suppl. Continued from the previous page

<table>
<thead>
<tr>
<th>Species</th>
<th>Most Preferred Prey</th>
<th>Reference(s)</th>
<th>Notes</th>
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<tbody>
<tr>
<td><em>Pipistrellus pygmaeus</em></td>
<td>Most preferred prey: small Nematocera, mainly Chironomidae and Ceratopogonidae, Culicidae, Simulidae, also Coleoptera, Trichoptera etc</td>
<td>Bartonička et al. 2008</td>
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<tr>
<td><em>Plecotus auritus</em></td>
<td>Mostly Lepidoptera, Coleoptera, Trichoptera, Neuroptera, Brachyptera, Dermaptera, Diptera, Arachnida</td>
<td>Swift &amp; Racey 1983; Vaughan 1997; Safi &amp; Kerth 2004; Andreas et al. 2012</td>
<td>√</td>
</tr>
<tr>
<td><em>Vespertilio murinus</em></td>
<td>Chironomidae, Diptera, Lepidoptera, Hemiptera</td>
<td>Rydell 1992; Safi &amp; Kerth 2004</td>
<td>√</td>
</tr>
<tr>
<td><em>Plecotus austriacus</em></td>
<td>Mostly Lepidoptera, Diptera too</td>
<td>Vaughan 1997; Safi &amp; Kerth 2004</td>
<td></td>
</tr>
<tr>
<td><em>Barbastella barbastellus</em></td>
<td>Lepidoptera, Diptera, few spiders, plant remains</td>
<td>Vaughan 1997; Safi &amp; Kerth 2004</td>
<td>found in nearby Switzerland</td>
</tr>
<tr>
<td><em>Rhinolophus ferrumequinum</em></td>
<td>Lepidoptera, Coleoptera</td>
<td>Vaughan 1997; Safi &amp; Kerth 2004</td>
<td>found in nearby Switzerland</td>
</tr>
<tr>
<td><em>Nyctalus leisleri</em></td>
<td>Mainly Scatophaga stercoraria, Scarabeoidea, Acari but also less Ephemeroptera, Trichoptera, Chironomidae, Ceratopogonidae, Culicidae, mainly Diptera, Muscidae, Tipulidae, Anisopodidae, Coleoptera, Lepidoptera</td>
<td>Vaughan 1997; Shiel et al. 1998; Safi &amp; Kerth 2004; Kanuch et al. 2005</td>
<td>found in the broad area and in Switzerland</td>
</tr>
</tbody>
</table>

19 10 11 11
CHAPTER V

Seasonal insect emergence from three different temperate lakes

Ioanna Salvarina, Dorian Gravier, Karl-Otto Rothhaupt

Manuscript for publication

ABSTRACT

Knowing the aquatic resources, such as emerging insects, that are entering terrestrial systems is important for food web studies and conservation plans, especially when water availability or quality is limited. Insect emergence from lakes in general, is less studied than emergence from rivers and freshwater benthic macroinvertebrates.

To understand if water parameters (e.g., water temperature, oxygen concentration etc) determine insect emergence and the possible seasonal differences, we collected emergent insects from three different lakes in South Germany, during three seasons. We searched for common patterns of insect emergence at the three lakes. To assess the relative contribution of insects of aquatic origin to other aerial flying insects, we also collected aerial flying insects at the shore.

Chironomidae constituted the highest number of emerged insects in all lakes, however different patterns of emergence occurred in each lake (unimodal vs. bimodal) with different times for the emergence peaks (spring, summer, beginning of autumn). Aquatic insects constituted a considerable proportion (at least 17%) of the aerial flying insects at the shore. The variation in insect emergence was explained by water temperature but not by other water parameters or the nutrient values. Seasonal and spatial differences in insect emergence should be considered when investigating aquatic-terrestrial interactions or designing conservation plans. We also provide length-dry weight relationships for emerged (adult) Chironomidae. These equations are useful to estimate dry insect biomass from length data.
INTRODUCTION

Material, energy, nutrient and organism fluxes can cross ecosystems boundaries and enter food webs of adjacent recipient systems in the form of subsidies. Subsidies are described as donor-controlled resources (prey, detritus, nutrients) from a given habitat to a recipient, where they can increase its productivity (Polis et al. 1997). Allochthonous inputs can: increase food-chain length (Pimm & Kitching 1987), influence food-web stability (Huxel & McCann 1998; Takimoto et al. 2002), and energy, carbon, and nutrient flow in a recipient system (Polis et al. 1997).

The emergence of insects from lakes is an example of subsidies that occur between aquatic and terrestrial systems. The life history of many aquatic insects includes larval stages in aquatic environments and an adult stage in terrestrial systems, therefore, movements across habitats occur. These emergent aquatic insects are important food sources for a great number of consumers, such as spiders, lizards, and several avian and mammalian species (e.g., Sabo & Power 2002a,b; Akamatsu et al. 2004; Fukui et al. 2006; Uesugi & Murakami 2007). However, there is a gap of knowledge in the assemblage and emergence patterns of these insects, especially from lakes and how these patterns relate to the lake’s trophic condition. Variation in insect community composition can occur in a relatively shorter spatio-temporal scale, allowing aquatic insects as a useful tool in monitoring the ecological status of a lake by examining emerging insects. Moreover, studying the patterns of insect emergence provide important data on the availability of this essential prey resource to adjacent systems.

Water warming can have an impact on aquatic insect emergence phenology (Greig et al. 2012) and, indirectly, could also influence terrestrial food webs. Additionally, aquatic bodies in many areas of the world are limited or affected by organic pollution. Knowledge on aquatic-terrestrial trophic interactions could help set priorities in conservation policies of animals and water bodies. Aquatic ecosystems are also the principal dietary sources of highly unsaturated fatty acids for all animals (Gladyshev et al. 2009). Insect emergence constitutes an important export of aquatic biomass, including fatty acids, to terrestrial ecosystems. Studies that estimate the fluxes of these highly unsaturated fatty acids, which play a key role to all organisms’ health, from particular aquatic ecosystems to surrounding terrestrial ecosystems and organisms are needed (Gladyshev et al. 2009).

Traditionally, research focuses on subsidies from terrestrial to aquatic systems, rather than the opposite direction (e.g., Polis et al. 1997; Finlay & Vredenburg 2007). Also, trophic relations between lentic ecosystems and terrestrial consumers have been less studied (e.g., Mehner et al. 2005; Kraus 2010), than lotic systems (e.g., Kawaguchi & Nakano 2001; Sabo & Power 2002a,b). Thus, our aim was: i) to assess the aquatic subsidies (from lakes) in the adjacent terrestrial system,
and ii) to search for general patterns in insect emergence at three different lakes, of the same region, in respect to season and water parameters such as temperature, oxygen concentration, pH, and nutrients. We collected emerged aquatic insects during spring, summer and autumn at three lakes (increasing in size and decreasing in trophic condition), in South Germany. Further, we addressed questions on the availability of these aquatic insects to terrestrial systems by looking into aerial flying insects, in spring and summer. To estimate and compare the biomass of aquatic insect subsidies from the three lakes, we calculated length-weight relationships for the most common aquatic insect taxa.

**MATERIALS AND METHODS**

**Study sites**

The study was conducted at three lakes in South Germany (Fig. 1) that differ in size and trophic status. Lake Constance is a deep (max. depth 254 m), large (500 km²), pre-alpine, oligotrophic (low in nutrient content) lake, situated in between Germany, Switzerland, and Austria. Our sampling site (47°41’27.72”N, 9°12’08.18”E) was in Upper Lake Constance, at the littoral zone with less than 10 m depth (Baumgärtner et al. 2008). Mindelsee (47°45’06.95”N, 9°01’24.80”E) is a shallower (max. depth 12 m), smaller, mesotrophic to eutrophic lake, included in a nature reserve. Siechenweiher (47°41’47.33”N, 9°16’.54.09”E) is a shallow (max. depth 2.5 m), highly eutrophic (http://www.seenprogramm.de/), small (about 0.05 km²) fishing pond at the edge of the town of Meersburg. The study was conducted during 2 years in order to have data covering the three ‘growing’ seasons (spring, summer, autumn).

**Insects**

Emerging aquatic insects were collected using, pyramid-like shape, floating traps (surface: 2500cm²: 50x50cm), with a bottle on the top filled with killing solution (either alcohol 80% or 1 alcohol: 1 ethylene glycol: 1 tap water). The traps were constructed at the University of Konstanz with model similar traps used in other studies (e.g., Hagen & Sabo 2012). Three to five traps were placed in Lake Constance (at about 1 m, 2 m, 3 m, 6 m, 8 m- the water level varied about 175 cm) and three traps in the other lakes (at 1 m, 2 m, 5 m in Mindelsee and at 1 m, 1.5 m and 2 m in Siechenweiher). The traps remained on the water from May to October 2011 and from April to June 2012. On average, insects were sampled every 5 days, with some variation due to logistic issues (e.g., bad weather). Between those samplings, three times per month (at each lake) we also collected insects that solely emerged at night (from sunset till sunrise). Hereafter, we refer to
emerged insects collected during the 5 days and nights as ‘day emerged insects’ and to those emerged during the night only as ‘night emerged insects’.

**Fig. 1 a.** Map of Europe where the study area is indicated with an arrow and, **b.** of the region with the lakes (upper left) and the sampling location in each lake: Constance, Mindelsee, Siechenweiher.

Aerial flying insects were caught using one Malaise trap constructed at the Limnological Institute, University of Konstanz (built with model the trap from Bioform®; 295x175x94 cm). The insects were trapped in a killing solution (either alcohol 80% or 1 alcohol: 1 ethylene glycol: 1 tap water) in the top and bottom (ground) of the trap. This trap was set up at the shore, near the floating traps, and was placed in a randomly orientation to avoid bias due to wind or possible corridors of flying insects. The Malaise trap, due to logistical constrains, was set up only 3 hours before the sunset, to give a picture of insects flying during the day. At sunset the first insect sample was collected and this corresponded to the ‘day aerial insects’. The next sample was collected the next morning at the sunrise time and corresponded to the ‘night aerial insects’ (from sunset to sunrise). The aerial flying insect collection was conducted only during April-June 2012.

In the laboratory, the insects were counted and classified to order or family level (based on: Roth 1974; Borror et al. 1989; Nilsson 1996, 1997). Diptera, mainly Chironomidae, Simulidae, were identified to family level. The body length, excluding antennas and other appendages, of the insects was measured under a microscope (10x magnification), in mm. Based on body length the insects were classified into four size groups (mm±0.1): <3; 3-6; 6-9; >9 mm.
For the calculation of the dry biomass of the emerged insects we oven dried (at 50°C for 48 hours to constant mass) and then weighted (to mg±0.001) using a microbalance subsamples of the three most abundant taxa, i.e., Chironomidae, Simulidae and Trichoptera from each lake, season and size class whenever possible. To get the dry biomass the median weights of Chironomidae from each size class, season and lake were then multiplied by the number of the total Chironomidae of that size class in each sample (Baumgartner & Rothhaupt 2003). For Trichoptera the median per lake was used and for Simuliidae the median from Lake Constance specimens was used. For other Diptera and the remaining taxa we used the weight of the second size group (3-6 mm) of Chironomidae of the respective lake and season each time.

For aquatic insects, emergence rate was calculated as number of individuals trap⁻¹ day⁻¹ for the insects collected during the 5 days and nights and individuals trap⁻¹ hour⁻¹ for the night insects. Due to the different night length through seasons we preferred to express the insect emergence per hour. We expressed the insects collected during the 5 days and nights as individuals per day as this is easier to conceive as opposed to the very low numbers per hour. For the emerged insect emergence, the mean values from all samples/traps of the same day(s) were used for the data analysis, unless some samples were destroyed or lost. The emergence rate of biomass was expressed as g trap⁻¹ day⁻¹ or hour⁻¹, using the mean values obtained from all the samples/traps of the same day(s).

Water parameters and nutrients

Once a month, during the data collection period, we collected water sample from each lake (at about 30 cm depth from total 1 m depth). The sample was taken in the morning and was transported to the lab and filtered. We stored the filtered water and the filter in the freezer till further analysis. Soluble reactive phosphate, nitrate and silicon were measured in the filtered water and particulate phosphorus and nitrogen in the filter. The analyses were done in a “Technicon AutoAnalyzer II”. Also after each night insect collection, physical and chemical parameters, i.e., temperature, dissolved oxygen, and pH, were also measured in the water about 30 min after the sunrise, using a temperature/oxygen portable device.

Statistical analysis

We tested for relationship between the day and the night emerged insects and between day and night insects (separately), the air temperature (at the time of sunset, from www.wunderground.com) and the water parameters (temperature, oxygen concentration, pH, silicon, phosphorus and nitrates) per lake. The normality of the data set was tested with the Shapiro test. To test for differences between day and night aerial insects, we used the Mann
Whitney Wilcoxon test, and the Kruskal-Wallis test for differences in the day emerged insects between seasons (per lake) and between lakes (per season). To test for differences between the day aquatic insect emergence between the different traps and subsequently different depth of the water column (trap location) we did Kruskal-Wallis tests per season and per lake. All analyses were performed using the statistics package R version 3.0.3 (R Core 2014) run within R Studio interface R version 0.98.932 (R Studio 2013). Additional packages that were used were: pgirmess (Giraudoux 2014) for Kruskal-Wallis test, gridExtra (Auguie 2012) and ggplot2 (Wickham 2009) for plots and maps, and ggmap (Kahle & Wickham 2013) for maps.

RESULTS

From a total of 561 samples, we counted 18,924 emerged aquatic insects. We could identify at least 14 different taxa in family or order level (Table 1), and some insects were unidentified. The vast majority was Chironomidae (82.4%); 55% of them were grouped to the size group of 3-6 mm, 26% of them to the size group of lower than 3 mm, and 18% to the size group of 6-9 mm. The night emerged insects were positively related to the day emerged insects at Lake Constance ($R^2=0.648$, $F=38.61$, $df=21$, $p<0.001$) and Mindelsee ($R^2=0.303$, $F=6.077$, $df=14$, $p=0.03$) (both ln(x+0.1) transformed). The number of day and night emerged insects per trap had a positive correlation with the biomass of day and night emerged insects respectively ($R^2=0.785$, $F=1371$, $df=375$, $p<0.001$, and $R^2=0.657$, $F=347.1$, $df=181$, $p<0.001$ respectively). Therefore, we used only emerged insect numbers for the further analysis (e.g., in tests for the relationship between water parameters and nutrients, for the plots).

The insect emergence showed seasonal fluctuations with different patterns in each lake. In Lake Constance and in Siechenweiher, there were two peaks noted: one in spring and one in the end of summer/autumn and in spring/beginning of spring and autumn, respectively (Fig. 2). In Mindelsee the peak was noted in summer (Fig. 2). Seasonal fluctuations were determined mostly by Chironomidae fluctuations. Different groups of insects had different peaks, for example Simuliidae emerged in high numbers in August in Lake Constance but were almost absent the rest of the time and in the other two lakes; Trichoptera were present in summer in Lake Constance and in summer and autumn in Mindelsee (Fig. 3). The numbers of day and night emerged insects differed only between between Lake Constance and Siechenweiher in summer ($p<0.02$) (Fig. 3). Kruskal-Wallis test: chi-squared=7.82, $df=2$, $p=0.02$ (Fig. 3). Differences in the insect emergence exist also between traps at different depths of the water column (Fig. 2). However, these differences were significant only in Mindelsee in spring and summer (Kruskal-Wallis test: $df=2$, $p<0.05$, spring: chi-squared= 9.909 and summer: chi-squared= 5.474).
The night emerged insects had positive correlations with the water temperature in Lake Constance and Siechenweiher (Fig. 4). There was no correlation between emerged insects and the other water parameters (oxygen concentration, pH, nutrients), except of night emerged insects and silicon in Siechenweiher where there was a positive relationship ($R^2=0.97$, $p=0.015$). The length-weight relationships that we calculated for the emergent Chironomidae differed between lakes and seasons but in all lakes the relationships were positive, although weak (Fig. 5 and Table 2).

**Table 1:** Mean ± se day insect emergence rate (individuals day$^{-1}$ trap$^{-1}$) during the period May-October 2011 and April-June 2012, and mean ± se number of aerial flying insects (before sunset) caught in the Malaise trap (individuals 3 hour$^{-1}$ trap$^{-1}$) in April-June 2012, separately for each lake. Note that emerged and aerial flying insect numbers are not comparable because the traps are different, cover different sampling area and the samplings were done during different times.
Fig. 2 Temporal emergence rate of insects (individuals day\(^{-1}\) trap\(^{-1}\)), for each lake. Smoothing was
done with loess method. Each color represents samples from traps at different depths. The depths
where the traps were located were: in Lake Constance: 1: 1 m, 2: 2 m, 3: 3 m, 4: 6 m, 5: 8 m; in
Mindelsee: 1: 1 m, 2: 2 m, 3: 5 m, and in Siechenweiher 1: 1 m, 2: 1.5 m and 3: 2 m.

Fig. 3 Boxplots of the most abundant insect taxa (Chironomidae, Simuliidae, Trichoptera)
emergence (individuals day\(^{-1}\) trap\(^{-1}\)) per lake and per season.

We counted 2567 aerial flying insects from 24 samples of the Malaise trap at the three lakes
that were identified in 14 taxa (Table 1). The most abundant groups in all three lakes were
Coleoptera (31% of total) and Chironomidae (16%). Terrestrial origin was attributed to 43% of all
aerial flying insects, 17% had aquatic origin (mainly Chironomidae) and the rest were not
attributed to aquatic or terrestrial origin. The day aerial insects per hour (caught during the 3
hours before the sunset) were significantly more than the night aerial insects (from sunset to
sunrise) (p<0.021 in all lakes). The aerial flying insect number was increasing with time in all lakes (Fig. 6).

The water temperature, pH, oxygen concentration and nutrient values per lake, and per season are reported in Table 3. The highest phosphate was found in Siechenweiher, the hypertrophic lake, while high nitrate was characteristic of the oligotrophic Lake Constance. Phosphate values were not significantly different between lakes; silicon was different only between Mindelsee and Siechenweiher, and nitrate between all pairs.

**Fig. 4** Night emerged insects (individuals hour\(^{-1}\) trap\(^{-1}\)) related to the water temperature at the sunrise (both ln(x+0.1) transformed). \(R^2\) and p values are also printed for each linear regression.

**Fig. 5** Length-weight relationships for emergent adult Chironomidae from each lake. Samples collected in each season are noted with different symbols and colours: blue points: summer, red triangles: spring, and green diamonds: autumn. The parameters for the regressions are given in Table 2.
DISCUSSION

We investigated the aquatic insect emergence at three lakes during three seasons. In general, calculations of insect emergence are relatively few, especially in larger scales. Bartrons et al. (2013) calculated the inputs of emergent aquatic insects to the terrestrial system in a regional-level and found that total emergence (lentic + lotic) was estimated to be about 6,800 metric tons of carbon per year. Benthic invertebrate communities are more often investigated than emerging insects. Fewer studies exist that assess both and most concern streams, for instance Poepperl (2000) estimated that the emerged biomass from streams in North Germany was between 1.0 and 2.0 g dry mass m⁻² year⁻¹ and was dominated by Diptera.

We recorded a seasonal pattern of insect emergence, typical for temperate regions and related to temperature (Corbet 1964). As is often the case in freshwaters, (e.g., Ivković et al. 2013; Čmrlec et al. 2013) the highest amount of emerged insects was Diptera, belonging mainly to Chironomidae family. In an older study in Mindelsee, Chironomidae comprised 75% of the total insect emergence in July-September 1984 (Smukalla & Meyer 1988) in agreement to 71% for the same months in Mindelsee during our study. The mean emergence rate for 1984 (calculated using data from Smukalla & Meyer 1988 and converted to same surface as our traps) was almost double than for the same months in our study (0.45 vs. 1.04 insects hour⁻¹ trap⁻¹). These differences in the percentage of Chironomidae and the total emergence rates could be due to inter-annual variations, differences in temperature, changes in benthic community or in fish predation as they consist prey for some fish species (Richeux et al. 1992; Bobori et al. 2013).

Differences in the presence of taxa between lakes might be related to lake characteristics, such as habitat structure and trophic condition. Hypertrophic conditions are often associated with higher abundance of macroinvertebrates than less eutrophic systems (e.g., Schell & Kerekes 1989). However, Siechenweiher, the most eutrophic lake of the study, had the lowest insect emergence per trap. Possibly, insect larvae were more abundant but were not allowed to emerge due to high fish predation, as Siechenweiher is used for fishing and is often stocked with fish. Fish predation probably was even more intense in spring, due to reproduction, possibly explaining the lower values of emerged insects in spring both in Siechenweiher and Mindelsee. On the contrary, the littoral zone of Lake Constance and our sampling location are characterized by low fish predation, which is most likely why insects emerged in high numbers.

Differences in the length-weight relationships among seasons or lakes are probably attributed to different species composition in each lake. However, identification in lowest level is needed for that, which was not the aim of this study. The length-weight relationships that we calculated can be used for estimations of dry insect biomass from the same lakes or other with similar biotic and
abiotic conditions. However, this should be done with care due to the differences we found between the lakes. The length-weight relationships that were calculated for benthic macroinvertebrates from Lake Constance have been found to differ from equations for the same taxa from streams (Baumgärtner & Rothhaupt 2003).

The number of aerial flying insects caught with the Malaise trap was higher than that of the emerged aquatic insects at the same time, however, different traps have different catching efficiency for different insect taxa and the data they produce are not comparable (Malicky 2002). Malaise trap is considered reliable in studying Chironomidae community structure over streams (Diserud et al. 2013).

Water temperature explained the variation in insect emergence rates. This is in accordance with other studies (e.g., Čmrlec et al. 2013). The other water parameters and nutrient values do not seem to play an important role in the insect emergence, therefore we do not see an effect of the nutrient content and trophic condition of the lake to the insect emergence. Water samplings, however, were less frequent than insect samples, and possible temporal differences in the water parameters might have affected the differences in insect emergence between lakes. Seasonal differences were also found in the benthic community in Lake Constance (Baumgärtner et al. 2008). Habitat structure might play an important role in the composition of the benthic community, which is related to insect emergence as well. Differences in the benthic macroinvertebrate communities were found for the littoral zone of Lake Constance between different depth zones mostly due to different substrates (Baumgärtner et al. 2008) and this might also be the reason for the differences we found in the emerged insects from different depths.

The aerial flying insect data show that a considerable amount (17%) of the insects, that are available as prey to insectivorous aerial flying consumers in spring and early summer, have aquatic origin. This might fluctuate seasonally however we do not have enough data to draw conclusions.

We found seasonal and spatial variation in the emergence rate. These results suggest that when aquatic fluxes to terrestrial systems are investigated, samplings should be done seasonally as a high variation in these fluxes might exist between different times of the year. Our insect emergence results can be used for models, such as that of Gratton & Vander Zanden (2009), of aquatic fluxes on terrestrial systems in a regional scale.
Table 2. Parameters for length-dry weight relationships of adult Chironomidae for each season and all seasons together, per lake. The relationship is: Dry weight=a+b*Length, where a: y-axis intercept, b: slope, SE: standard error, $R^2$: coefficient of determination, range of length: min-max of length (mm) and weight (mg).

<table>
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<th>Lakes</th>
<th>Season</th>
<th>n</th>
<th>a+SE</th>
<th>b+SE</th>
<th>$R^2$</th>
<th>range of length (mm)</th>
<th>range of weight (mg)</th>
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<td>-0.032±0.057</td>
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<td>0.285</td>
<td>24–74</td>
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<td>summer</td>
<td>10</td>
<td>-0.091±0.040</td>
<td>0.004±0.001</td>
<td>0.713</td>
<td>24–65</td>
<td>0.0012–0.2704</td>
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<td>-0.047±0.030</td>
<td>0.003±0.001</td>
<td>0.421</td>
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<td>13</td>
<td>-0.010±0.060</td>
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<td>0.377</td>
<td>24–84</td>
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<td>0.0388–0.3883</td>
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<td>0.015±0.001</td>
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<td>spring</td>
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<td>0.633±0.742</td>
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<td>0.010</td>
<td>29–83</td>
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<tr>
<td></td>
<td>all seasons</td>
<td>25</td>
<td>-0.009±0.056</td>
<td>0.003±0.001</td>
<td>0.077</td>
<td>12–83</td>
<td>0.0087–1.7625</td>
<td>0.180</td>
</tr>
</tbody>
</table>

Table 3: Water parameters (water temperature, oxygen concentration, pH) and nutrients (P: phosphate, N: nitrate, Si: silicon) per lake and per season. Mean values ± standard deviation. Lakes: CO: Constance, MI: Mindelsee, SI: Siechenweiher.

<table>
<thead>
<tr>
<th>Season</th>
<th>lake</th>
<th>water T (°C)</th>
<th>oxygen (mg/l)</th>
<th>pH</th>
<th>P (µg/l)</th>
<th>N (µg/l)</th>
<th>Si (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>spring</td>
<td>CO</td>
<td>10.52±3.12</td>
<td>10.00±2.63</td>
<td>8.32±0.08</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>15.71±4.15</td>
<td>9.26±1.12</td>
<td>8.36±0.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>SI</td>
<td>16.92±4.82</td>
<td>7.91±1.68</td>
<td>7.98±0.29</td>
<td>5.8</td>
<td>10</td>
<td>338</td>
</tr>
<tr>
<td>summer</td>
<td>CO</td>
<td>19.20±2.28</td>
<td>8.79±2.05</td>
<td>8.30±0.14</td>
<td>2.83±0.17</td>
<td>697.75±67.92</td>
<td>686.50±219.37</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>21.91±1.73</td>
<td>8.79±1.40</td>
<td>8.27±0.15</td>
<td>3.50±1.04</td>
<td>400±138.04</td>
<td>515.67±141.33</td>
</tr>
<tr>
<td></td>
<td>SI</td>
<td>21.61±3.05</td>
<td>6.26±2.69</td>
<td>7.64±0.48</td>
<td>5.40±1.04</td>
<td>46±31.32</td>
<td>1352.67±367.77</td>
</tr>
<tr>
<td>autumn</td>
<td>CO</td>
<td>16.05±3.30</td>
<td>9.47±1.63</td>
<td>8.21±0.29</td>
<td>6.60±1.84</td>
<td>674.00±111.72</td>
<td>720.00±87.68</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>18.05±0.21</td>
<td>11.08±0.11</td>
<td>8.43±0.25</td>
<td>6.2</td>
<td>156</td>
<td>576</td>
</tr>
<tr>
<td></td>
<td>SI</td>
<td>14.68±2.81</td>
<td>8.25±2.22</td>
<td>7.55±0.35</td>
<td>8.7</td>
<td>21</td>
<td>1583</td>
</tr>
</tbody>
</table>
Fig. 6 Aerial flying insects during 3 hours before sunset (individuals hour$^{-1}$ trap$^{-1}$), for each lake.

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We thank BUND (Bund für Umwelt und Naturschutz Deutschland) and Kai-Steffen Frank, and the Angelsportverein Meersburg e.V. for allowing us to conduct fieldwork in Mindelsee and Siechenweiher, respectively. Many thanks for the technical assistance to Christian Fiek, Martin Wolf, Alfred Sulger and Josef Halder, and for help in the field to Salman Said and Markus Köpf. We also thank Hans-Günther Bauer and Kamran Safi for useful discussions during the designing of the project, Elizabeth Yohannes for constant support with advices and for reviewing the manuscript, and Paul Preston for the language corrections. IS is a member of the International Max Planck Research School for Organismal Biology and she is thankful for the stipend she received from it.
GENERAL DISCUSSION & CONCLUSIONS

This Ph.D. thesis investigates aquatic-terrestrial interactions, using as a model the relationship between bats and aquatic insects. The studies that are included in this dissertation apply two independent, yet complimentary, methodological approaches: bio-chemical tracers (stable isotopes and fatty acids analysis on faeces), and a field study conducting bat acoustic monitoring in tandem with insect collections from three lakes. The Ph.D. thesis was completed with a literature review on the use of aquatic resources by bats. This review aims to highlight the importance of conservation, particularly the effects that anthropogenic impacts on freshwater systems have on bats.

Aquatic-terrestrial trophic interactions and bats’ use of aquatic habitats

One important subject in ecology is food web dynamics, and this dynamic becomes more complex (and interesting) when the food web extends across different ecosystems’ boundaries. Terrestrial animals may use aquatic food resources according to their feeding habits, their energy requirements, and availability of other prey. Consequently, the importance of aquatic resources for terrestrial consumers might exhibit temporal, spatial, or interspecies differences. Knowing the aquatic subsidies for terrestrial consumers is essential to understand the life history patterns of organisms that rely on the interface of the two ecosystems. Additionally, this knowledge can be of use for management plans at the species and ecosystem level, and for conservation, e.g., to predict possible effects of climate change across systems. The importance of studying aquatic-terrestrial subsidies is even greater due to the fact that many water bodies are suffering from pollution that can transfer through the food web to terrestrial consumers. Aquatic insects can be vectors for pollutants to terrestrial consumers. Indeed, it has been shown that, near hydroelectric reservoirs, insectivorous bats have significantly higher concentrations of mercury in their fur compared to frugivorous bats (Syaripuddin et al. 2014). Knowing the relationship between bats and aquatic bodies is also helpful for understanding the distribution of species and biodiversity that are important for ecology and conservation. For example, temperature and water availability are key factors that determine elevational bat species richness (McCann 2007).

The systematic literature review (Chapter I, Synopsis in Fig. 1 of this section) showed that there is both direct and indirect evidence (150 studies found) that aquatic bodies are important habitats for bats, and often attract higher bat activity than other habitats in the area. Most studies are concentrated in limited regions, so there are hardly any intensive studies in regions such as Africa, Asia, and South America. Even studies in Europe are not homogenously distributed, and fail to cover important areas equally. This review also shows the need for more studies on arid
environments. The lack of comparable studies on the indirect effects of anthropogenic activities on bats via aquatic bodies prevents from drawing any concrete conclusions. This is especially true for the effects of eutrophication, although most disturbances tend to have a negative effect (Table 3, Chapter I). The characteristics of a water body that attract bats differ between species, habitat, and regions. However, the literature review indicates a rather clear preference of bats for non-cluttered waters (Table 2, Chapter I).

The characteristics of a water body that attract bats differ between species, habitat, and regions. However, the literature review indicates a rather clear preference of bats for non-cluttered waters (Table 2, Chapter I).

![Diagram](image-url)

**Bats**

- Use
- Feed on

**Bats**

- pollution, eutrophication
- characteristics of aquatic systems: pond/river width, water system size, riparian vegetation, smoothness of the water surface, hydroperiod, insect availability, altitude, water availability
- season
- animals’ energy requirements, sex, age, species

**influence bats’ use of aquatic resources**

**bats’ dependence on aquatic resources is studied using**

- acoustic monitoring, radio-tracking, mist-netting, stable isotopes, molecular analysis, visual identification of faeces, visual observations (video, camera), experiments

**aquatic systems:**
- rivers, streams, canals, lakes, ponds, sea, coastal areas, artificial reservoirs, wetlands. riparian areas

**aquatic insects (Diptera, Trichoptera, Ephemeroptera)**
- mosquitoes, fish, shrimps, sea lion blood, frogs

**Fig. 1** Synopsis of the review on the use of aquatic resources by bats.

The review also showed that, like the general tendency for studies dealing on aquatic-terrestrial subsidies, there are few studies for lentic systems compared to lotic. The direction of subsidies from terrestrial to aquatic systems is also more studied than the reverse. However, in the case of bats, the studies on aquatic-terrestrial interactions are only conducted with the objective of studying bats, and not focused on the bats’ influence on aquatic systems. Top-down effects of bats on aquatic systems and bat foraging effects on aquatic or terrestrial food webs, e.g., by suppressing insect populations or limiting available prey for other terrestrial consumers, are rarely investigated. However, the indirect effects of insectivorous bats on tropical and temperate forests via insect consumption have been studied (e.g., Morrison & Lindell 2012 and Böhm et al. 2011, respectively). This is likely due to the value of applying such studies in forestry and agriculture; for instance, insectivorous bats play an important role in controlling herbivorous insects and their damage to trees.
Although terrestrial predators other than bats, such as birds, lizards, and arthropods, are known to feed on emerging aquatic insects (reviewed by Baxter et al. 2005), there are no investigations on the whole food web and the interactions that take place. Large-scale experiments that consider multiple aquatic and terrestrial components of the food web are rare. These experiments, though difficult to conduct, might reveal new aspects of aquatic–terrestrial trophic interactions, such as competition for aquatic resources between bats and other terrestrial insectivores.

Not many studies cover all of the seasons when bats are active. Results of this Ph.D. thesis emphasize the importance of considering seasonal variations in bat activity (Fig. 2, Fig. 3 Chapter IV) and insect emergence (Fig.2, Fig. 3, Chapter V). Field data collected in this study indicate that seasonality and temperature variations are important factors influencing bat activity. The study was carried out during one temperate (continental European) ‘bat year’ (three seasons, excluding winter when bats are usually hibernating). The winter diet of bats is understudied, but there is evidence that M. nattereri has an altered foraging strategy, feeding on non-volant prey in winter (Hope et al. 2014). This is something to consider in future studies.

Additional important questions in ecology are how animals make decisions related to foraging and space occupancy and how competition and niche partitioning take place. Studies of bat communities, such as those in this thesis (Chapter IV), and not of single species, may shed more light on the complex interactions between species and other parameters.

Investigation of bats’ use of aquatic resources using non-invasive methods: bio-chemical tracers and acoustic monitoring

The use of non-invasive methods as ecological tools can be very helpful when endangered species are concerned. We used stable isotopes of carbon, nitrogen and sulphur, and omega-3 and omega-6 fatty acids on faeces. Our results from the feeding experiment on captive bats showed that a shift in the diet is reflected in faeces’ stable isotope within 2-3 hours (Fig. 1, Chapter II). Faeces can be used as non-invasive, cost-efficient samples for stable isotope analysis, especially when the research question focuses on recent and/or rapid shifts in habitat and/or diet. The values of the isotopic differences between diet and faeces, that were calculated in the controlled experimental study (Table 1, Chapter II), are valuable information for reconstructing diet using only faecal samples obtained from field studies. The little variation that was found between the two bat species, both insectivorous, suggests that these results can be applied to other insectivorous bat and small mammal species. However, this should be done with caution, as differences in diet, water stress, or species metabolism/physiology might cause the values to vary.
The stable isotope analysis on wild bat faeces proved the hypothesis that faecal isotopic signatures differ between bats according to the aquatic versus terrestrial origin of their consumed food (Fig. 2, Chapter III). Similarly, fatty acid markers on the same faecal samples allowed us to discriminate between the species (*Myotis daubentonii*) that feeds on an aquatic diet from the species (*M. myotis*) that feeds on a terrestrial diet (Fig. 4, Chapter III). Interestingly, the species *M. mystacinus*, which is known to have both an aquatic and terrestrial diet, had intermediate values of both stable isotopes and fatty acids. These results showed that both stable isotopes and fatty acids in bat faeces are reliable methods to investigate resource transfer between systems. This approach could well be extended to study similar research questions not only in freshwater vs. terrestrial systems, but also between marine vs. freshwater or terrestrial systems, between agricultural areas vs. urban areas, and among systems differing in altitude, latitude or levels of pollution.

An important finding was also the utility of the less often used sulphur stable isotope. This can be helpful to reveal heterogeneity in the diet or the habitat use that is not obvious with only the two more common stable isotopes, i.e. carbon and nitrogen. Stable sulphur can be especially useful when animals feed on marine habitats, as it is related to salinity (Fry & Chumchal 2011).

Microscopic visual identification of prey remains in faeces usually cannot confirm the habitat origin of the prey. This is an advantage of stable isotopes and fatty acid methods, provided that the habitat signatures are significantly different. However, these chemical tracers still cannot tell us the prey species. Molecular analyses can identify the exact species, if the species DNA is known. The acoustic monitoring that we used together with emergent insect collections is an indirect way of showing potential aquatic-terrestrial relations. One of the advantages of acoustic monitoring is that it can be passive and large datasets can be easily collected. Moreover, data can be analysed efficiently using automatic software that has been developed for the calls of bat species of some geographical regions. Complimentary use of bio-chemical tracers with other methods, such as molecular or microscopic faeces analysis or acoustic monitoring, can yield better information of the animals’ diet and/or foraging habitat.

We studied the seasonal variations of insect emergence from three different lakes in the same region. These data can be used, in the future, for estimations of total aquatic subsidies in the adjacent terrestrial systems. This is the first study of insect emergence from Lake Constance, one of the largest lakes in Europe that has a large drainage basin and provides water for thousands of people. The current study is also one of the few dealing with insect emergence from lakes in Germany (except Smukalla & Meyer 1988). More studies exist for rivers than for lakes, and for freshwater benthic invertebrates than for emerging insects, not only in Germany, but also worldwide (e.g., Baumgärtner et al. 2008; Poepperl 2000).
In general, we did not find strong correlations between bat activity and emerging aquatic insects (Fig. 5, Table 1, Chapter IV), especially in one of the lakes (Mindelsee). Fluctuations in bat activity could be explained by seasonality or other factors such as habitat characteristics. Another possible reason why insect emergence did not explain bat activity could be that food resources were always abundant, or the recorded species might feed partly or entirely (Table 1, Supplement, Chapter IV) on terrestrial food. It was not possible to discriminate species that feed exclusively on aquatic prey from species that feed on terrestrial or both. Indeed, aerial flying insects showed some positive correlation with bat activity of species that are known to feed on both terrestrial and aquatic prey (Table 1, Chapter IV).

The nocturnal pattern of bat activity (Fig. 4, Chapter IV) also has interest for ecological research and conservation. Different species might shift their nocturnal patterns to avoid competition, especially under limited food resources. Generally, the emergence time of bats from roosts has been more widely studied than the pattern of bat activity throughout the night.

We found seasonal and spatial variations in the nocturnal patterns of total activity. From our results, there are two interesting outcomes. Firstly, regardless of the study site, bat activity was not limited to only after sunset or before sunrise. Bats can be active during the whole night, however, with only acoustic monitoring, we cannot know if the same bats are active in the same area. Second, the bat activity pattern during the night can vary at different lakeshores, despite the fact that they are in the same region and climatic conditions and bat species are the same. There was no general pattern that could be described as representative of the bats in this region. This is perhaps because microclimate or habitat differences might play a role. Additionally, the availability of other foraging habitats in the area might contribute to the variability in nocturnal activity patterns. Since we did not measure nocturnal insect activity, it is not possible to relate bat activity patterns with insect availability. Our results indicate that ecologists and conservationists should consider these seasonal and spatial variations in bat activity when studying interactions between species or monitoring bat activity in an area.

**Outlook and future directions**

The results of the projects in this Ph.D. thesis show the seasonal variations of aquatic subsidies in terrestrial systems and highlight the overall importance of aquatic insects and lakes for bats. Stable isotope and fatty acid analyses on bat faeces are shown to be successful ecological tools to study aquatic contributions in the diet of terrestrial consumers, such as bats, and possibly other small mammals.

The question as to what extent terrestrial consumers depend on aquatic resources is important, especially since nowadays species distributions are changing due to climatic changes,
often expanding to warmer places with less water. Thus, for conservation, it is essential to know the resilience and plasticity of species to dietary shifts, or the changes in prey availability. To study the flexibility of bats on shifting between diets, we can use stable isotopes on faeces from individuals that are caught while returning, probably several times per night, to their night roosts. As faecal stable isotope and fatty acid signatures reveal short-term diet and habitat information, it is possible to investigate how bats use different habitats in the same region and on the same night.

Future directions of research could include investigations on whether aquatic insects, food that is rich in omega-3 fatty acids, give bats some advantages, or are related to more complicated behavior or hunting mechanisms. Deficiencies in omega-3 fatty were related to behavioural disorders, in mammals (Brenna 2011).

Large-scale controlled field experiments, excluding aquatic insect emergence or terrestrial insects, might reveal how bats react to these resource limitations and whether they shift to another type of prey. However, this might differ according to the energy requirements of bats at the specific time. Field studies or experiments applying multiple methods might reveal more detailed information on the use of aquatic resources by bats. For example, video recordings can compliment acoustic monitoring to estimate the number of different bats flying at the same time, or tracking of individuals might reveal how they distribute their time among different habitats.

For ecological studies, but also conservation, especially concerning endangered species, it is essential to investigate competition for aquatic insects between bats and other terrestrial consumers (e.g., birds, lizards, arthropods). It would also be interesting to do similar studies in drier areas, where aquatic resources might be a limiting factor, and possibly predict and even prevent the indirect effects of climate change on these regions.
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CONTRIBUTIONS

General Introduction and Discussion: entirely my own work. Some of my Ph.D. advisory committee members and other colleagues provided useful feedback.

Chapter I: the idea was mine and it is exclusively written by me with feedback from other people mentioned in the acknowledgements.

Chapter II: the project was designed by me, Elizabeth Yohannes, and Björn Siemers with advice from my Ph.D. advisory committee. The experiment was conducted by me and Klemen Koselj. The samples were prepared by me. The data and statistical analyses were done mainly by me, with help from Klemen Koselj. The paper was written by me with help from the coauthors.

Chapter III: the project was designed by me and my Ph.D. advisory committee. The sample collection and their preparation was done by Monika Lam (under my supervision). The data and statistical analyses for the paper were done by me and Monika Lam. The paper was written by me and Monika Lam, with input from the other coauthors.

Chapter IV: the project was designed mainly by me with the help of my Ph.D. advisory committee. The fieldwork in 2011 was done from me (with help from student assistants) and in 2012 from me and Dorian Gravier. The bat call analysis was done by me. The insect counting and identification was done by me (for 2011) and Dorian Gravier (for 2012). Most plots were done by me, with help from Dorian Gravier. The manuscript was written by me, with comments from the coauthors.

Chapter V: the project was designed mainly by me with the help of my Ph.D. advisory committee. The fieldwork in 2011 was done from me (with help from student assistants) and in 2012 from me and Dorian Gravier. The insect counting and identification was done by me and Dorian Gravier. The data analysis and plots were done by me with feedback from Dorian Gravier. The manuscript was written by me, with comments from the coauthors.
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Published in peer-reviewed journals


Submitted manuscripts

Salvarina I, Koutrakis E & Leonaridos I. Comparative study of feeding behaviour of five Mugilidae species juveniles from two estuarine systems in the North Aegean Sea (in revision for Journal of the Marine Biological Association of the United Kingdom)

Salvarina I, Gravier D & Rothhaupt K-O. Seasonal bat activity related to insect emergence at three temperate lakes (in review in Journal of Mammalogy)
Salvarina I. Bats’ use of aquatic habitats: a review emphasizing how anthropogenic impacts on water bodies affect bats (in review in Mammal Review)

**Manuscript in preparation for publication**

Salvarina I, Gravier D & Rothhaupt K-O. Seasonal insect emergence from three different temperate lakes

**Contributions in international conferences**

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Salvarina I, Yohannes E, Gravier D & Rothhaupt K-O (2012) Aquatic subsidies (emerging insects) to terrestrial consumers (bats). ASLO Aquatic Sciences Meeting. Lake Biwa, Japan (oral presentation)


Salvarina I, Koutrakis M & Leonardos I (2010) Juvenile feeding habits of Mugilidae species from estuarine systems in North Aegean Sea. 39th CIESM Congress. Venice, Italy (poster presentation)


Bobori DC & Salvarina I (2007) Fish species composition and abundance in the Greek part of Lake Doirani. 1st Symposium for protection of the natural lakes in FYROM. Ohrid, FYROM (poster presentation)