

**Assessment of bat activity in agricultural environments and the
evaluation of the risk of pesticides**

**Untersuchungen zur Fledermausaktivität in landwirtschaftlichen
Habitaten und eine Abschätzung des Risikos durch Pestizide**

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Campus Landau

Vorgelegt von Peter Stahlschmidt

Gutachter:

1. Prof. Dr. Ralf Schulz

2. Prof. Dr. Martin Entling

(Institut für Umweltwissenschaften Universität Koblenz-Landau, Campus Landau)

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1 SUMMARY

1.1 English Summary

Although agriculture dominates with around 50% area much of Europe's landscape, there is virtually no information on how bats use this farmed environment for foraging. Consequently, little is known about effective conservation measures to compensate potential negative effects of agrarian management practice on the food availability for bats in this habitat. Moreover, there are currently no specific regulatory requirements to include bats in European Union risk assessments for the registration of pesticides since no information about pesticide exposure on this mammal group is available.

To evaluate the potential pesticide exposure of bats via ingestion of contaminated insects, information about bat presence and activity in agricultural habitats is required. In order to examine bat activity on a landscape scale it was necessary to establish a suitable survey method. Contrary to capture methods, telemetry, and direct observations, acoustic surveys of bat activity are a logistically feasible and cost-effective way of obtaining bat activity data. However, concerns regarding the methodological designs of many acoustic surveys are expressed in the scientific literature. The reasons are the failing of addressing temporal and spatial variation in bat activity patterns and the limitations of the suitability of the used acoustic detector systems. By comparing different methods and detector systems it was found that the set up of several stationary calibrated detector systems which automatically trigger the ultrasonic recording has the highest potential to produce reliable, unbiased and comparable data sets on the relative activity of bats.

By using the proposed survey method, bat diversity and activity was recorded in different crops and semi-natural habitats in southern Rhineland-Palatinate. Simultaneously, the availability of aerial prey insects was studied by using light and sticky traps. In more than 500 sampling nights about 110,000 call sequences were acoustically recorded and almost 120,000 nocturnal insects were sampled. A total of 14 bat species were recorded, among them the locally rare and critically endangered northern bat (*Eptesicus nilssonii*) and the barbastelle (*Barbastella barbastellum*), all of them also occurring over agricultural fields.

The agricultural landscape of southern Palatinate is dominated by vineyards, a habitat that was shown to be of low quality for most bat species because of the demonstrated low availability of small aerial insects. By surveying bat activity and food availability in a pair-wise design on several rain water retention ponds and neighbouring vineyards it was demonstrated that aquatic insect emergence in artificial wetlands can provide an important resource subsidy for bats. The creation of artificial wetlands would be a possibility to create important foraging habitats for bats and mitigate negative effects of management practice in the agricultural landscape.

In several other agricultural crops, however, high abundances of suitable prey insects and high bat activity levels, comparable or even higher than in the nearby forests and meadows known to be used as foraging habitats were demonstrated. Especially high bat activity levels were recorded over several fruit orchards and vegetable fields where insects were also present. Both crops are known for high pesticide inputs, and, therefore, a pesticide exposure through ingestion of contaminated insects can not be excluded.

To follow the current risk assessment approach for birds and mammals pesticide residues were measured on bat-specific food items in an apple orchard following insecticide applications and bat activity was recorded in parallel. The highest residue values were measured on foliage-dwelling arthropods which may result in a reproductive risk for all bat species that, even to a small extent, include this prey group in their diet.

The presence of bats in agricultural landscapes that form a majority of the land area in Europe but also on a global scale leads to exposure of bats by contaminated food and depletion of their food resources by pesticide use. So far conservation efforts for bats focussed on securing hibernation sites and the creation of artificial roost sites since especially the latter were thought to be limiting population growth. However the potential pesticide effects might be also crucial for the population persistence in agricultural landscapes of bats and need to be addressed adequately, especially in risk assessment procedures for the regulation of pesticides.

1.2 German Summary (Zusammenfassung)

Etwa 50 % der Fläche Europas werden landwirtschaftlich genutzt. Dennoch gibt es nahezu keine Information ob Fledermäuse diese Flächen beispielsweise zur Nahrungsaufnahme nutzen. Aufgrund der limitierten Datenbasis mangelt es auch an Schutzkonzepten, die mögliche negative Effekte der landwirtschaftlichen Intensivierung auf die Fledermäuse und deren Nahrungsgrundlage ausgleichen könnten. Da die Exposition von Fledermäusen mit Pflanzenschutzmitteln bislang nicht thematisiert wurde, sind im europäischen Zulassungsverfahren für Pflanzenschutzmittel keine Risikoabschätzungen für Fledermäuse gefordert.

Um für Fledermäuse die mögliche Exposition gegenüber Pflanzenschutzmitteln abschätzen zu können, sind Informationen über Vorkommen und Aktivität von Fledermäusen in landwirtschaftlich genutzten Flächen erforderlich. Die Erfassung von Fledermäusen auf einer Vielzahl von Flächen machte es notwendig sich im Vorfeld auf eine geeignete Methodik festzulegen. Die akustische Fledermauserfassung ist im Gegensatz zu deutlich zeitaufwändigeren Methoden wie Netzfang, Telemetrie oder direktes Beobachten die einzige logistisch durchführbare Methode. In der wissenschaftlichen Literatur werden jedoch bei vielen bisher durchgeführten akustischen Methoden Bedenken bezüglich der Berücksichtigung zeitlicher und räumlicher Varianz und der Eignung der verwendeten Detektorsysteme geäußert. Deshalb wurden verschiedene Methoden und Detektorsysteme verglichen und das parallele Beprobieren mit mehreren stationären und kalibrierten automatischen Aufnahmesystemen als die am besten geeignete Methode zur verlässlichen und vergleichbaren Fledermausaktivitäts-Erfassung befunden.

Mit dieser Methode wurden die Fledermaus-Diversitäten und Aktivitäten in verschiedenen landwirtschaftlichen Kulturen, Wiesen und Wäldern aufgenommen. Außerdem wurde gleichzeitig die Verfügbarkeit von fliegenden Insekten (potentieller Fledermausbeute) mit Licht- oder Klebefallen erfasst. In mehr als 500 Erfassungsnächten wurden circa 110,000 akustische Fledermaus-Rufsequenzen und nahezu 120,000 nachtaktive Insekten gesammelt. Insgesamt wurden 14 Fledermausarten nachgewiesen, darunter die im Gebiet sehr seltene und stark bedrohte Nordfledermaus (*Eptesicus nilssonii*) und die Mopsfledermaus (*Barbastella barbastellum*). Alle Arten wurden auch auf landwirtschaftlich genutzten Flächen detektiert.

Die Landwirtschaft im südlichen Rheinland-Pfalz ist durch Weinanbau geprägt. Die Untersuchungen zeigten, dass Weinberge aufgrund der geringen Verfügbarkeit an kleineren nachtaktiven Insekten für die meisten Fledermausarten nur eine geringe Qualität als Jagdgebiet haben. Ein weiterer paarweiser Vergleich von Weinbergen und benachbarten Regenrückhaltebecken bezüglich Nahrungsverfügbarkeit und Fledermausaktivität zeigte, dass Regenrückhaltebecken wichtige Nahrungshabitate im Weinbaugebiet darstellen. Das Anlegen

dieser künstlichen Kleingewässer ist somit eine geeignete Methode um Nahrungshabitate für Fledermäuse zu schaffen und damit den negativen Effekten der konventionellen Landwirtschaft hinsichtlich der Nahrungverfügbarkeit entgegenzuwirken.

In anderen landwirtschaftlichen Kulturen wurden mit den parallel untersuchten Wald- und Wiesenhabitaten vergleichbar hohe Insektenvorkommen und Fledermausaktivitäten nachgewiesen. Besonders hohe Fledermausaktivitäten so wie eine besonders hohe Verfügbarkeit von geeigneten Nahrungstieren wurden in Apfelplantagen und Gemüsefeldern gemessen. Da diese beiden Kulturen hohem Pestizidaufwand unterliegen, kann eine Exposition von Fledermäusen gegenüber Pflanzenschutzmitteln dort nicht ausgeschlossen werden.

Um das zurzeit verwendete Verfahren zur Risikoabschätzung von Pflanzenschutzmittelanwendungen auf Vögel und Säuger auf Fledermäuse zu übertragen, wurden nach Applikation eines Insektizides dessen Rückstände auf Fledermaus-artspezifischen Beuteinsekten gemessen. Parallel dazu wurde die Fledermausaktivität erfasst. Die höchsten Pestizidrückstände wurden auf kronenbewohnenden Insekten und Spinnen nachgewiesen. Die darauf basierende Risikoabschätzung deutet auf ein Langzeitrisiko für alle Fledermausarten, die sich wenigstens zum Teil von kronenbewohnenden Arthropoden ernähren, hin.

Das Vorkommen von Fledermäusen in landwirtschaftlichen Flächen, die einen Großteil der europäischen, aber auch der weltweiten Fläche ausmachen, führt je nach Kultur zur Exposition von Fledermäusen durch kontaminierte Nahrung oder zu einer Verringerung von Beuteinsekten. Bisher konzentrierten sich Schutzbemühungen auf die Sicherung von Winterquartieren und die Bereitstellung künstlicher Tagesquartiere. Potentielle Effekte von Pflanzenschutzmitteln auf Agrarflächen haben aber möglicherweise einen entscheidenden Einfluss auf die Populationsgrößen von Fledermausarten die in diesen Gebieten vorkommen. Aus diesem Grund sollten die Effekte von Pflanzenschutzmitteln auf Fledermäuse untersucht werden, insbesondere bei der Risikoabschätzung im Pflanzenschutzmittel-Zulassungsverfahren.

2 BACKGROUND

2.1 Bats

Bats are summarizing a group of mammals, with more than 1,200 extant species worldwide (Schipper *et al.*, 2008). The combination of flight and echolocation set bats apart from other mammals and permit them access to a wide range of habitats and resources at night. As a result they occupy almost all terrestrial habitats in most climatic zones and exploit a great variety of foods ranging from arthropods, vertebrates, and blood to fruits, leaves, pollen and nectar (Kunz & Pierson, 1994).

Bats have long been postulated to play an important role in arthropod suppression, seed dispersal, and pollination, but only recently there is an increasing awareness of their ecosystem services (Kunz *et al.*, 2011). Given their high energy demands, insectivorous bats need to consume tremendous amounts of arthropods, reported as being up to more than 100% of the body weight per night (Kurta *et al.*, 1989). Considering the high food demand and the fact that various species of prominent agricultural insect pests have been found in the diets of bats, their importance in agricultural pest suppression was demonstrated (Cleveland *et al.*, 2006; Boyles *et al.*, 2011; Kunz *et al.*, 2011). Boyles *et al.* (2011) even argued that bats are among the most overlooked economically important, non-domesticated animals. Moreover, because bats fill such a wide array of ecological niches, they offer an important multisensory role in assessing ecosystem health and, therefore, have a great potential as suitable bioindicators (Jones *et al.*, 2009).

2.2 Bat populations under threat

Bats, however, are also among the most endangered vertebrates in the world, with 22% of the species considered as threatened and another 23% listed as near threatened (Hutson *et al.*, 2001). Especially in Central Europe where 29 bat species occur (Dietz *et al.*, 2007), they have undergone serious population declines since the mid 20th century. Once among the most widespread and abundant bat species of Central Europe, the lesser horseshoe bat (*Rhinolophus hipposideros*) is today extinct in many regions (Bontadina *et al.*, 2008). But even species still considered to be fairly common such as the widespread common pipistrelle (*Pipistrellus pipistrellus*) have declined in recent years with a decrease in abundance of over 62% between 1978 and 1993 according to roost counts in Great Britain (Hutson, 1993).

As a consequence all bats, their resting and breeding sites are strictly protected in Europe under the European Habitats Directive (Conservation of Natural Habitats of Wild Flora and Fauna 1992/42/EEC) (Stone *et al.*, 2012).

Various anthropogenic activities have contributed to the decline of the European bat species. Several bat species that in the past only occupied tree cavities and caves, which have become scarce due to deforestation and urbanisation, now regularly use man-made structures as roost sites. However, suitable manmade structures such as deserted buildings and barns are now being converted into modern buildings at rapid rates (Kunz & Reynolds, 2003) and, additionally, bats roosting in buildings are often not welcome and face exclusion (Jones *et al.*, 2009). The disturbances of hibernating bats by cave tourism can lead to death (Kunz *et al.*, 2011). Moreover, human infections are more likely to be spilled over to bat populations with increasing contact to humans (Jones *et al.*, 2009). In North America, the White-Nose Syndrome caused by the fungal pathogen *Geomyces destructans*, which was probably introduced from Europe, has already lead to substantial declines in hibernating bats (Wibbelt *et al.*, 2010). Roads have a major negative impact on bats, because of vehicle collision, habitat fragmentation and disturbance by noise (Schaub *et al.*, 2008; Berthinussen & Altringham, 2011). Light pollution has been shown to negatively affect bat foraging and roosting behaviour (Stone *et al.*, 2012). Bats may also be negatively affected by heavy metal pollution since several studies have reported significant concentrations of e.g. mercury in bats (O'Shea & Johnson, 2009). Recently, the development of wind energy facilities has also caused extensive mortality of bats (Kunz *et al.*, 2007) and climate change may affect the distribution of bats (Sachanowicz *et al.*, 2006; Jones *et al.*, 2009). Agricultural intensification, however, is considered as the major cause of the observed declines in European bat populations during the second half of the 20th century (e.g. Stebbings, 1988; Wickramasinghe *et al.*, 2004, Bontadina *et al.*, 2008; Jones *et al.*, 2009).

2.3 The impact of agricultural intensification on bats

In Europe agriculture is the dominating land-use, covering nearly half of the EU members states' surface area (Stoate *et al.*, 2001). There is increasing evidence that, in recent decades, intensification has contributed to the impoverishment of farmland biodiversity (e.g. Sotherton, 1998; Krebs *et al.*, 1999; Donald *et al.*, 2001; Stoate *et al.*, 2001). The negative impact has been noted across many different species groups including wildflowers (Kleijn & Snoeiijing,

1997), insects (Aebischer, 1991; Benton *et al.*, 2002), birds (Krebs *et al.*, 1999), and bats (Wickramasinghe *et al.*, 2003).

Agricultural intensification takes places at different scales. At the landscape scale, the replacement of traditional, less extensive mixed farming systems by large and homogeneous arable fields has caused major alteration or even losses in non-crop habitats such as hedgerows, field margins and wetlands. Habitat deterioration acts through a decrease in the ecological quality while habitat loss extinguishes valuable foraging, shelter and breeding sites of bats, as well as reduces the availability of habitats for their prey (Jones *et al.*, 2009). Moreover, habitat fragmentation caused by habitat destruction may lead to a separation of populations and severely impact population dynamics (Andren, 1994). For bats the role of hedgerows, which have been lost on a large scale due to the enlargement of field sizes (Robinson & Sutherland, 2002), has been pointed out as commuting routes (Verboom & Huitema, 1997), dispersal corridors (Walsh & Harris, 1996), and, since they serve as shelterbelts where swarms of insect congregate (Lewis & Dibley, 1979), as important foraging habitats. Wetlands have frequently been converted in arable land through drainage measures, which has resulted in a loss of up to 90% of wetland area in the intensively cultivated regions of Europe (e.g. Mitsch & Gosselink, 2000; Thiere *et al.*, 2009). By supporting large numbers of insects, wetlands are among the most important foraging habitats for most insectivorous bat species (e.g. Vaughan *et al.*, 1997; Scott *et al.*, 2010).

At the local field scale the intensification of agriculture was facilitated through increased mechanization and the use of synthetic agrochemicals such as fertilizers and pesticides. Declines of insects have been linked to the use of pesticide (e.g. Campbell *et al.*, 1997). Since all Central European bats are insectivorous, declines in insect abundance as a result of agricultural intensification are likely to have serious implications for bats. Moreover, bats are reported as being directly threatened by pesticides (O'Shea & Johnson, 2009; Jones *et al.*, 2009). Evidence of direct effects of agricultural pesticides on bats was recognized in the 1960s and 1970s, a period of widespread use of organochlorine pesticide. Some of these pesticides were responsible for significant mortality of several bat species as demonstrated by laboratory and field studies in Europe and northern America (e.g. Jefferies, 1972; Gelusco *et al.*, 1976; Clark *et al.*, 1978). It was demonstrated that bats taken from one of the most intensively farmed areas of Great Britain were more heavily contaminated with residue levels of organochlorine than birds (Jefferies, 1972). Moreover, laboratory experiments demonstrated that bats were more sensitive to these pesticides than were other mammals (Jefferies, 1972; Jones *et al.*, 2009). Lipophilic pesticides such as organochlorine can have

detrimental effects by accumulating in the stored fat. When fat is metabolized during hibernation or migration, pesticide concentration can reach high and toxic levels, especially in the brain (Clark, 1988). Thus, bats carried one-third of the lethal levels, but this rose to lethal levels following hibernation (Jefferies, 1972; Jones *et al.*, 2009). More recently, a die-off of juvenile greater mouse eared bats (*Myotis myotis*) was documented after the application of Filitox (active substance: methamidophos), an organophosphate to potato fields and apple orchards in Germany (Hoffmann, 1991). The high levels of methamidophos residues detected in the corpses were considered to be transferred through milk to the offspring by females which consumed contaminated insects. In Spain, residues of organophosphates were reported in common pipistrelles (*Pipistrellus pipistrellus*) following agricultural applications (Guillén *et al.*, 1991). Today, most of the highly toxic and persistent pesticides have been replaced and therefore the effects of modern pesticides on bats may be more difficult to document, have been less well studied and are probably underappreciated (O'Shea & Johnson, 2009).

2.4 Strategies to improve the situation for bats in agricultural landscapes

The pesticide risk assessment for bats and the identification of risk mitigation measures as well as effective conservation strategies to improve the situation for bats in agricultural landscapes are urgent issues.

Highlighted by Rachel Carson's book *Silent Spring* (1962), there was growing public awareness about the potentially harmful effects of environmental chemicals, especially pesticides, to both human and wildlife in the 1960s (Walker, 2006). Responding to these developments, research institutes undertook programmes to investigate side effects of environmental pollutants (Walker, 2006). The need for assessing the ecotoxicological risk of pesticides on non-target organisms was also recognized by the regulatory agencies such as the European Food and Safety Authority (EFSA). No authorisation is granted for new pesticides unless a risk assessment demonstrates that no risk for wildlife species occurs when the pesticide is applied under field conditions (EFSA, 2009). This procedure also includes a risk assessment on birds and mammals (EFSA, 2009). Risk is estimated by applying a number of different generic indicators ("generic focal species"), which are not real species, however, regarding their feeding habits, representative for species that occur in a particular crop at a particular time (EFSA, 2009). Insectivorous mammals are only represented by the generic indicator "shrews" but no reference is made to bats. However, bats differ widely from other mammals in their feeding habits and also in their ecological traits such as the physiological

constraints due to hibernation and migration and the low reproductive rates (usually a single offspring per year) which can render bat populations highly susceptible to effects of pesticides. The reason for the missing implementation of bats in the risk assessment approach is probably related to the limited knowledge about the occurrence and activity of bats in agricultural crops and herewith the uncertainties about the potential pesticide exposure of bats.

Since agricultural intensification has already led to an alarming level of ecological degradation, there are now more and more efforts to improve the landscape heterogeneity with ecological compensation programs. Organic farming is a production system in which the use of synthetic fertilizers and pesticides are largely excluded. By demonstrating higher abundance of nocturnal insects and higher activity levels of bats on organic farms compared to conventional farms it was suggested that bats benefit from organic farming (Wickramasinghe *et al.*, 2003; Wickramasinghe *et al.*, 2004; Fuller *et al.*, 2005). Agri-environment schemes such as the management of field margins, hedgerows, water margins and species-rich grasslands have been introduced as an attempt to reverse biodiversity declines caused by agricultural intensification (e.g. Baker *et al.*, 2012). Also wetland creation in the agricultural landscape with the aim to improve ecosystem services like nutrient, pesticide and water retention but also biodiversity has recently received much attention (Stehle *et al.*, 2012; Thiere *et al.*, 2009). In order to incorporate the requirements of bats into the different schemes, more research is needed to examine if and how they benefit bats.

3 OBJECTIVES AND THESIS LAYOUT

The primary objective addressed in my thesis was to assess to which extent agricultural fields and orchards are used as foraging habitats by bats. This framework evolved from the missing implementation of bats in the current European pesticide risk assessment approach which is caused by the limited knowledge about the occurrence and activity of bats in agricultural crops and uncertainties about their pesticide exposure.

In order to accomplish this objective, four main parts were identified and studied (see also Fig.1):

- **Method establishment:** in order to examine bat activity patterns on a landscape scale it was necessary to establish an acoustic survey method that fulfilled the needs of a standardized, quantitative recording to produce reliable, unbiased and comparable data sets on bat activity [Appendix 1]. In the course of the study it became necessary to get insights in flight patterns of bats flying in small scaled habitats. It was tested if a combined detection field of several simultaneously recording stationary bat-detectors has the potential to provide insights in flight patterns [Appendix 2].
- **Activity survey:** bat activity and prey availability were assessed in a multitude of different crop fields and orchards. In order to normalize the activity levels recorded in the examined agricultural fields we simultaneously recorded in forest and meadow habitats known to be attractive foraging areas [Appendix 3; Appendix 4].
- **Evaluating the benefit of a compensation measure:** based on the relatively low prey availabilities and low bat activity levels demonstrated in vineyards in the course of the survey part, the benefit of artificial wetlands as foraging habitats for bats in areas dominated by vineyards was evaluated [Appendix 5].
- **Risk assessment approach:** based on the high bat activity levels recorded in apple orchards in the course of the survey part, a field study was performed to measure pesticide residues on bat specific prey items. By using the toxicity-exposure ratio approach of the current European pesticide risk assessment, the residue values allowed us to estimate the risk for bats foraging in apple orchards [Appendix 6].

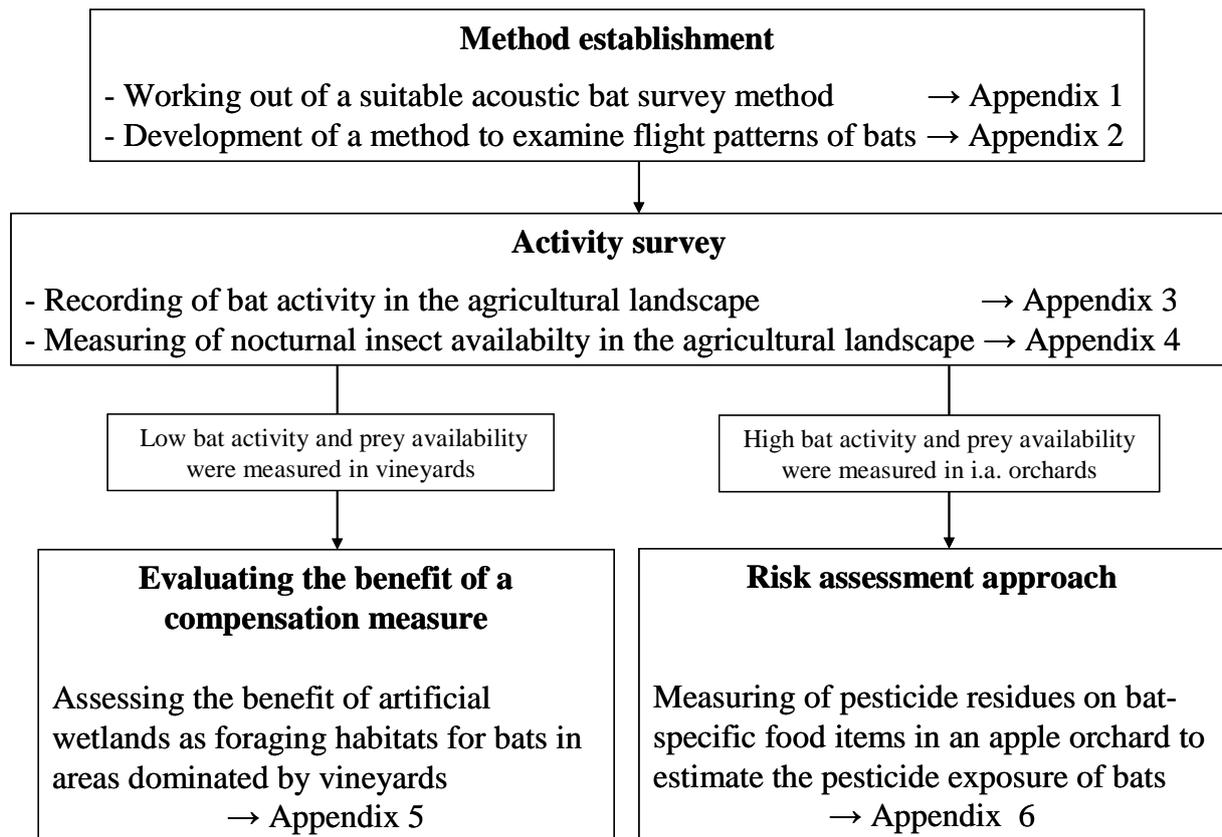


Figure 1. Flowchart indicating the processes and context of data generation and publications of the present thesis.

4 RESULTS AND DISCUSSION

4.1 Method establishment

For bat surveys, several methods such as acoustic techniques, capture methods, telemetry and direct observations are available, all differing in their limitations and advantages (Hayes *et al.*, 2009). However, even though a combination of several techniques to survey bats could result in deeper insights than one technique alone (MacSwiney *et al.*, 2009) the use of ultrasonic detectors is often the only logistically feasible way of obtaining bat activity data over a large study area and a long time period.

Detecting activity by the means of acoustic methods to assess the relative amount of use of a site by bats can be useful for making comparisons between sites, habitats, and the same site over a time period. However, Hayes (2000) and Sherwin *et al.* (2000) expressed fundamental concerns regarding the basic design of many previous acoustic studies due to the lack of replications, the failure to address spatial and temporal variation in activity pattern and the missing consideration of the equipment limitations. Gannon *et al.* (2003) showed that by disregarding those underlying conditions false predictive models could be generated.

Generally, there are two different acoustic bat survey methods: the transect walk and the stationary measurement. By conducting transect surveys and simultaneously using several stationary systems I measured bat activity within a homogenous habitat and evaluated which method assessed the spatial bat activity patterns within this habitat with highest precision [Appendix 1]. Our results indicate that the transect survey fails to represent the heterogeneous bat activity patterns in a homogenous landscape.

Acoustic detector systems can generally be divided in two ways of triggering the recording of ultrasonic signals: actively by a fieldworker or passively by a built-in recording control algorithm of an automatic device. I measured bat activity simultaneously and side by side with both methods for direct comparison and demonstrated occurring errors based on the subjective hearing of the human operator actively triggering the signals [Appendix 1].

In conclusion, the set up of several stationary sampling systems which automatically trigger the ultrasonic recording such as the batcorders (ecoobs) has the highest potential for standardized acoustic bat surveys [Appendix 1]. In the performed survey study of the present thesis 10 batcorders were used simultaneously in each study area per night [Appendix 3]. This study design with several sampling sites in different habitats grouped in a study area allowed

the direct comparison of activity levels between the different habitats under the same conditions (weather, season).

With acoustic detectors information on site specific bat activity can be obtained. However, this method fails if the research requires statements whether reported bat activity at a particular site is caused by foraging or commuting bats. Furthermore, acoustic surveys cannot be used to quantify number of bats in an area as it is not possible to distinguish between a single individual of a species passing the detection field of a detector several times and several individuals each passing it once (Hayes, 2000). It was demonstrated that combined detection fields of several simultaneously recording stationary bat detectors with overlapping detection fields have the potential to provide insights in individual flight patterns. This approach is a useful tool to examine if previously recorded bat activity in small scaled habitats (e.g. clearings) or along linear landscape elements (e.g. hedgerows) is caused by many bat specimens using the area as part of a transfer paths or by few bats species foraging there [Appendix 2].

4.2 Survey of bat activity and prey availability

A total of 14 bat species were recorded over agricultural crops in the course of the survey study (Table 1) [Appendix 3]. The activity levels recorded of the genera *Pipistrellus*, *Eptesicus* and *Nyctalus* (see Table 1 for the recorded species), all of them being predominately aerial hawkers (Dietz *et al.*, 2007), did not differ between forest and open landscape habitats. When comparing only the different open landscape habitats (agricultural and meadow habitats) significantly lower bat activity levels were recorded over vineyards. This coincided with the low abundances of suitable prey insects for these bat species found over vineyards [Appendix 3; Appendix 4].

For most *Myotis* species (see Table 1 for the recorded species) higher activity levels were found in the forests and significantly reduced activity in the open landscape habitats [Appendix 3]. Natterer's bat (*Myotis nattereri*) and Bechstein's bat (*Myotis bechsteinii*) are known to take their prey mainly and the whiskered bat (*Myotis mystacinus*) and Brandt's bat (*Myotis brandtii*) at least partly by gleaning from vegetation (Dietz *et al.*, 2007). The echolocation of bats using this foraging strategy is adapted to high-clutter environments but not to open landscape habitats (Aldridge & Rautenbach, 1987). Daubenton's bat (*Myotis daubentonii*) is adapted to take the prey from water surfaces (Dietz *et al.*, 2007). In contrast, highest average activity levels of the greater mouse-eared bat (*Myotis myotis*), a species

almost exclusively feeding on carabid beetles, were recorded over vegetable fields (Table 1) [Appendix 3]. The greater mouse-eared bats capture their prey from the ground and therefore select habitats such as vegetable fields that offer high accessibility to the ground (Arlettaz, 1999).

The grey long-eared bat (*Plecotus austriacus*) was almost exclusively recorded in vineyards (Table 1) [Appendix 3] which was in concurrences with the significantly higher abundances of moths of the family Noctuidae there [Appendix 4], the preferred prey of that bat species (Dietz *et al.*, 2007).

The barbastelle (*Barbastella barbastellum*) was recorded twice at forest edges and once in a forest (Table 1) [Appendix 3]. The occurrence of that rare species in the study area was the first record for southern Rhineland Palatinate since the species was only known from a single location in northern Rhineland Palatinate so far (König & Wissing, 2007).

Table 1. Occurrences of bats in the different examined habitat. On the basis of the total number of recorded passes of every bat species the percentile share of recorded passes per habitat was calculated and ranked in the following way: – : 0%; x: 0.1-5%; xx: 5.1-15.0%; xxx: 15.1-50.0%; xxxx: 50.1-100%. For further information on the data I refer to Appendix 3.

Species	forest	edge	meadow	vineyard	orchard	vegetable	cereal
<i>Pipistrellus pipistrellus</i>	x	xxxx	x	x	xxx	xx	x
<i>Pipistrellus nathusii</i>	x	xxx	xx	x	xxx	x	xx
<i>Pipistrellus pygmaeus</i>	xx	xxx	x	—	xxx	xx	xx
<i>Eptesicus serotinus</i>	xx	xxx	xxx	x	xx	xx	xx
<i>Eptesicus nilssonii</i>	xxx	xxx	xxx	x	x	xx	x
<i>Nyctalus noctula</i>	xx	xxx	xx	x	x	xx	xx
<i>Nyctalus leisleri</i>	xx	xxxx	xx	x	xx	x	xx
<i>Myotis mystacinus / brandtii</i>	xxx	xxx	x	x	xx	xx	x
<i>Myotis daubentonii</i>	xxx	xxx	xx	x	x	x	x
<i>Myotis bechsteinii</i>	xxxx	xxx	x	x	—	x	—
<i>Myotis nattereri</i>	xxx	xxx	x	xx	xxx	x	x
<i>Myotis myotis</i>	xx	xxx	x	xx	—	xxx	xx
<i>Plecotus austriacus</i>	xx	xxx	xx	xxxx	—	xx	xx
<i>Barbastella barbastellum</i>	xxx	xxxx	—	—	—	—	—

Our results of high activity of several species over agricultural crops appear in contrast with several studies that have found an avoidance of arable land (e.g. Walsh & Harris, 1996;

Vaughan *et al.*, 1997). However, habitats which are known as preferable foraging habitats for bats such as woodland edges and water habitats are rare within most European landscapes while in contrast arable land constituting more than 40 % of the available habitat (Walsh & Harris, 1996). Therefore, the predominant arable land, even if disproportionately more scarcely used by bats, may play an important and currently underestimated role as a foraging habitat. Wickramasinghe *et al.* (2003) compared bat activity across conventional and organic agricultural land and recorded higher activity on organic farms. However, these differences were only found over water but not over land habitats (Davy *et al.*, 2007). Fuentes-Montemayor *et al.* (2011) demonstrated even generally lower bat activity levels on farms involved in agri-environmental schemes than on conventionally-managed farms. Relatively large numbers of foraging attempts of bats were recorded in some arable fields in South Europe (Russo & Jones, 2003). Foraging activity was also reported in intensively cultivated olive orchards treated with insecticides (Davy *et al.*, 2007). Moreover, intensively managed apple orchards were documented as being positively selected as foraging habitats by the greater mouse-eared bat (Arlettaz, 1999; Drescher, 2004).



First picture (T. Stephan): The pipistrelle (*Pipistrellus pipistrellus*) the most common bat species recorded over agricultural habitats. Second picture (C. Brühl): Preparing a batcorder for recording of bat activity in a forest.

All examined bat groups showed remarkably high activity levels over agricultural fields located next to forests (Table 1) [Appendix 3]. The northern bat (*Eptesicus nilssonii*) and the barbastelle, both of them very rare species in Rhineland Palatinate (König & Wissing, 2007), were predominantly recorded at the forest edges. Due to their structure, forest edges are suitable habitats for aerial hawkers that avoid navigating through structurally complex habitats as well as those that avoid the open landscape such as gleaners. Moreover, forest edges function as windbreaks and can concentrate large densities of insects (Lewis, 1970). A

number of studies have already identified this habitat as preferred foraging areas for bats (e.g. Walsh & Harris, 1996; Morris *et al.*, 2010) but - to our knowledge - no data about the importance of forest edges next to agricultural fields are available. Given the limited number of examined sites in the course of the survey part [Appendix 3], a follow-up study was performed [data unpublished]. Three different forests were chosen that border on agricultural fields at one side and on meadows at the other side, allowing the direct comparison. At each forest, bat activity was recorded simultaneously at the edge to the field, the edge to the meadow and within the forest (clearing) at four occasions between May and August 2009. No differences in total bat activity was found between both edges types (paired *t*-test: $t = 0.985$, $P = 0.021$, $n = 3$) while less bats were recorded within the forest (Figure 2). Given that in agricultural landscapes most forest edges are situated next to crop fields, the importance of this habitat was demonstrated.

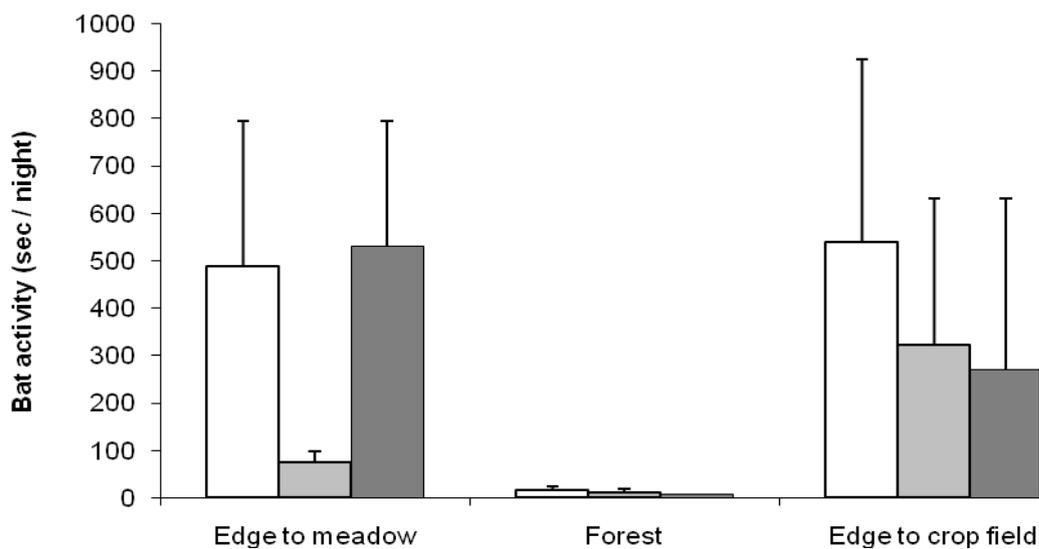


Figure 2. Bat activity pattern at three different forests (white, grey, dark grey). In every forest, activity was simultaneously recorded at three different habitats (edge to meadow, forest, and edge to crop fields) at three occasions between May and August. The means and the Standard Deviations are shown (data unpublished).

Many populations of insect species have markedly declined in the last decades, primarily as a result of agricultural intensification (e.g. Feber *et al.* 1997; Kromp, 1999), but very little is known about the impact of agricultural intensification on the aerial nocturnal insects eaten by bats (Wickramasinghe *et al.*, 2004). Benton *et al.* (2002) reported that diversity, abundance, and biomass of aerial insects are negatively associated with agricultural intensification.

Wickramasinghe *et al.* (2004) demonstrated higher nocturnal insect abundances on organic farms compared to conventional farms. However, the differences were found over pastures and water habitats, but not over arable land (Wickramasinghe *et al.*, 2004). Fuentes-Montemayor *et al.* (2011) found that overall insect abundances (about 70 % of them were Diptera) was almost twice as high on conventionally managed farms compared to farms involved in agri-environmental schemes. No differences in nocturnal insect availability were found between woodland, organic olive orchards, and orchards treated with insecticides (Davy *et al.*, 2007). Apart from the exception of lower overall insect availability over vineyards, no differences were found in abundances of nocturnal insects of the examined sizes classes between agricultural, forest and meadow habitats in the present study [Appendix 3].



First picture (C. Brühl): Preparing of a light trap in a cereal field. Second picture (M. Hahn): A typical catch of a light trap, parts of the moths were sorted to size classes.

I cannot provide any information on the differences in biodiversity of nocturnal insects trapped in the different crops and semi-natural habitats as they were not identified to species level (with the exception of Noctuidae, see discussion below). Only abundance of nocturnal insects were compared between habitats, however more than 70% of them belonged to the flies and midges (Diptera). In a study by Nielsen *et al.* (1994) the occurrence of Diptera was not significantly impacted by pesticide use and, while tillage has been reported as a disturbance factor for terrestrial Diptera, some species are even specialized on the initial stages of succession after tillage (Frouz, 1999). Thus some Diptera species may be less affected by agricultural intensification and occur in high abundances in the crop fields. The main factors affecting the occurrence of Diptera with terrestrial larval stages are the organic matter content and the moisture of the soil (Frouz, 1999). The soils of several crops are especially rich in organic matter due to the remnants of the former crops. Moreover, crops such as vegetables provide permanently wet soils due to irrigation. In contrast, vineyards do

not providing conditions very attractive for most Diptera since their soils are rather dry due to their exposed position.

Moths (Lepidoptera) in general and the family Noctuidae in particular, however, showed higher abundances and species richness in vineyards in comparison to the vegetable and cereal fields [Appendix 4]. A possible explanation may be the greater availability of host plants: In contrast to the arable crops which were regularly ploughed, at least 50% of the vineyards were covered with vegetation. Since the abundance of some moth species has been shown to correlate with the abundance of host plants (Saarinen, 2002), the area covered with vegetation might influence the moths community significantly. Another factor possibly influencing the abundance and diversity of Noctuidae are the different pesticide regimes in the crops. While in the vineyards of the study region the two most important pest species are targeted with pheromones and therefore no insecticides were applied, insecticides are commonly used in the arable fields (Roßberg, 2007) which might affect moths as well. Laboratory studies have documented lethal and sublethal effects (e.g. weight loss, feeding inhibition) on butterfly caterpillars after exposure to insecticides (Tan, 1981; Cilgi & Jepson, 1995).

On the one hand higher overall insect abundances were recorded in intensively cultivated crops treated with insecticides while on the other hand higher species richness for the only group examined, the noctuid moths, were demonstrated for vineyards that receive no insecticide applications and have a low soil management. Thus, it appears that several insect species, especially some Diptera, are relatively insensitive to agricultural intensification and can occur in high abundances and presumably being dominant elements of the species-poor insect communities of the intensively cultivated crop fields.

In summary, the presence of bats and suitable prey insects in agricultural crops were demonstrated, among them crops that require high pesticide inputs [Appendix 3; Appendix 4]. Thus, an uptake of pesticides through consumption of potentially contaminated food items after pesticide application cannot be excluded.

4.3 Evaluating the benefit of artificial wetlands in vineyards for bats

Agricultural intensification has led to an impoverishment of biodiversity in the agricultural environment (e.g. Benton *et al.*, 2002). Ecological compensation programs have been introduced as an attempt to improve the landscape heterogeneity and to counteract the negative effects of intensive agriculture. In order to incorporate the requirements of bats into the different schemes, the effects of the compensation programmes on foraging bats and

availability of prey insects have to be evaluated. Recently, Fuentes-Montemayor *et al.* (2011) demonstrated that pipistrelle bats and their prey do not benefit from widely applied agri-environment management prescriptions such as the management of field margins, hedgerows, water margins and species-rich grasslands.



First picture (L. Ressler): one of the examined retention ponds, with a line for sticky traps established 2 m above the water surface. Second picture (C. Brühl): Sticky traps at night to survey the nocturnal insect density of the retention pond.

Wetland creation in the agricultural landscape has also been applied as a compensation measure for past wetland losses and the benefit of these artificial wetlands for the biodiversity of invertebrates was pronounced by Thiere *et al.* (2009). Aquatic ecosystems are known as favourable foraging habitats for many bat species (e.g. Scott *et al.*, 2010; Vaughan *et al.*, 1997). Recent studies have demonstrated that in natural ecosystems aquatic insect emergence can provide an important resource subsidy for bats (Fukui *et al.*, 2006; Hagen & Sabo, 2011). It was shown that bats also use artificial water bodies such as sewage treatment works (Park & Christinacce 2006) or irrigation ponds and linear waterworks in a semiarid Mediterranean landscape (Lisón & Calvo, 2011). Given the low bat activity levels and availabilities of small prey insects in vineyards as demonstrated in the course of the field survey [Appendix 3; Appendix 4], it was examined if artificial ponds created for water retention in vineyards also benefit bats [Appendix 5]. Our results indicated that bat activity and nocturnal prey density were significantly higher above the retention-ponds, with total bat activity being at a factor of 16 to almost 200 higher above the retention-ponds (Figure 2). When relating foraging activity to habitat availability within an assumed home-range of 1.5 km of the common pipistrelle (*P. pipistrellus*), retention-ponds had on average the same importance as foraging habitat as the complete vineyard area, although covering less than 0.1% of its area [Appendix 5].

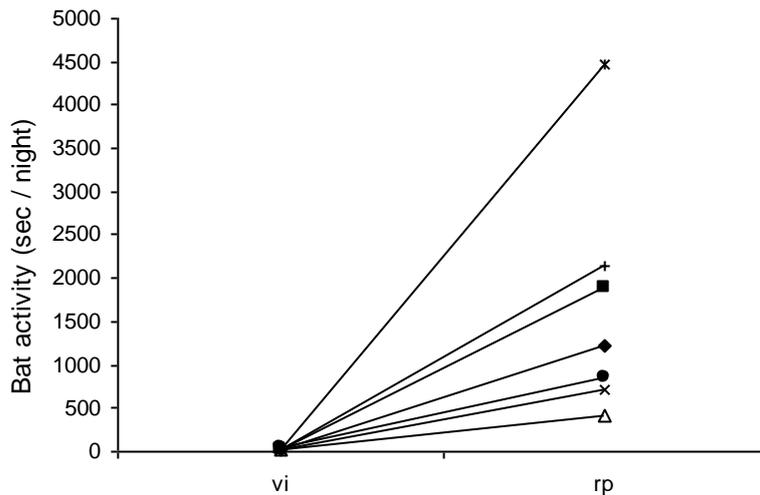


Figure 2. Differences of mean activity of bats between seven pairs of retention-ponds (rp) and vineyards (vi). Values shown are the means of activity in seconds per night (number of samplings nights: 8-9 for each site).

Thus, artificial ponds, although few in numbers and small in size, may be key spatial structures for several bat species in agricultural landscapes as they represent important foraging habitats. Habitat management for bats should include the creation of suitable artificial wetlands. In this way bat conservation can be combined with widely accepted land management activities for water or nutrient retention and environmental restoration measures with minimal land conversion and without additional costs.

On the other hand, aerial insects with aquatic larval stages have been reported as transmitters of contaminants from aquatic to terrestrial systems (e.g. Walters *et al.*, 2008; Park *et al.*, 2009). Especially chironomids, an important prey group for most bat species, are known to transfer substantial contaminant mass to terrestrial ecosystems because of their high productivity (Menzie, 1980; Walters *et al.*, 2010). The large discrepancy of concentrations detected in the sediment or water and in the aerial insects (approximately 700-folds) suggests that they even can highly accumulate chemicals (Park *et al.*, 2009). Insect-borne aquatic contaminants bioaccumulate in terrestrial spiders (Walters *et al.*, 2008), insectivorous birds (Custer *et al.*, 2003) and bats (Pikula *et al.*, 2010). Budd *et al.* (2011) evaluated the accumulation of pesticides within constructed wetlands in an agricultural area and demonstrated the persistence of pyrethroids and chlorpyrifos, with DT_{50} values (time for 50 % degradation) between 106–353 days under flooded conditions. Research would be required to examine if an accumulation of pesticides takes also place in insects developing in the artificial wetlands posing another exposure pathway for bats.

4.4 Risk assessment of pesticide exposure to bats

Given the high bat activity levels recorded at several agricultural crop fields and the availability of suitable prey insects [Appendix 3] an exposure to pesticides through consumption of contaminated prey is very likely. Especially high bat activity levels were recorded in several apple orchards, a crop known for high insecticide input (Roßberg, 2007). Since the estimation of the exposure requires information on pesticide residues on bat-specific food items, a follow-up study [Appendix 6] was performed in one of the apple orchards where high bat activity levels were demonstrated. According to the preferences of the recorded bat guilds the residue pattern of different nocturnal arthropod groups was examined following the application of the insecticide Fenoxycarb an insect growth regulator affecting larval stages of insects in their moulting phase. The highest residue values were measured on foliage-dwelling arthropods such as spiders and insects. The acute and reproductive risks were estimated by following the TER (toxicity-exposure ratio) approaches of the current European pesticide risk assessment (EFSA, 2009). The exposure was based on the concentration of the calculated pesticide residues in the species-specific diet, bat's body mass, and the food intake rate. For the toxicity component of the ratio calculation, the LD₅₀ (lethal dose; the dose where 50% of the test organisms die) of an acute oral test was used for the acute risk assessment, whereas the NOAEL (no observed adverse effect level) values of reproduction tests or multi-generation studies were used for the reproductive risk assessment. The LD₅₀ and NOAEL values used in the calculation were from rat and mice, respectively. It is common practice that risk assessments evaluating the hazard posed by pesticides on wildlife species are based on toxicities of a standard range of test species, such as laboratory rats, mice, rabbits, etc. To account for interspecific variability in sensitivity, the TER values are compared to safety factors (10 for acute and 5 for reproductive risk) in the European pesticide risk assessment. If the TER is larger than the safety factor, the risk is considered to be low. If the TER is lower than the safety factor, no authorization is granted for the pesticide unless a refined risk assessment demonstrates that no risk for wildlife species occurs when the pesticide is applied under field conditions (EFSA, 2009). By following that approach, no acute dietary risk was found for all recorded bat species. However, there is uncertainty if the applied safety factor of 10 used in the TER approach of acute toxicity accounts for interspecific variability in sensitivity (Luttik & Aldenberg, 1997; Hart *et al.*, 2001). Story *et al.* (2011) highlighted the importance of evaluating the effects of pesticides on species that are phylogenetically distinct from those used in the laboratory test to evaluate the toxicities. It was shown that Australian dunnarts (*Sminthopsis*; Mammalia: Marsupiala) were 10 to 14 times more sensitive than

similar sized eutherian mammals (Story *et al.* 2011). Given the high TER values (larger than 600, Table 2) obtained, an acute dietary risk of fenoxycarb appears unlikely as a risk would only be expected for species with at least 600 times higher sensitivities to this substance.

Table 2. Toxicity exposure ratios (TER) of fenoxycarb for several bat species and species groups based on their assumed diet compositions. The combinations of prey groups resulting in the lowest and highest values within the range of assumed species-specific diet compositions are shown. Bold values indicate that they are below the safety factor value (10 for acute risk assessment, 5 for reproductive risk assessment).

Species	Range of the assumed diet	TER _{acute}	TER _{repro}
<i>P. pipistrellus</i>	95% 'flying insects', 5% 'foliar dwelling arthropods'	5683.4	6.4
	90% 'flying insects', 10% 'foliar dwelling arthropods'	3651.4	4.2
<i>Myotis mystacinus</i>	50% 'flying insects', 50% 'foliar dwelling arthropods'	944.5	1.1
	40% 'flying insects', 60% 'foliar dwelling arthropods'	797.1	1.0
<i>Myotis nattereri</i>	30% 'flying insects', 70% 'foliar dwelling arthropods'	689.4	0.8
	20% 'flying insects', 80% 'foliar dwelling arthropods'	607.4	0.7
' <i>Nyctalus-Eptesicus</i> '	25% 'flying insects', 75% 'large moths'	22537.8	23.3
	75% 'flying insects', 25% 'large moths'	14854.4	16.0

However, a reproductive risk for bat species that include foliage-dwelling arthropods in their diet was indicated (Table 2) [Appendix 6]. The justification of the applied trigger value of 5 for reproductive risk assessment to account for between-species variation in toxicity has also been criticised (Luttik *et al.*, 2005). Bats may be especially sensitive to pesticides due to their ecological traits (De Lange *et al.*, 2009). They differ in many aspects from rodents commonly used in laboratory tests and also from shrews used as a surrogate for insectivores requiring high food intake rates. Most bat species have long lifespans and therefore more time for contact with, or accumulation of, dangerous levels of pesticides (Clark, 1988). Their low reproductive rates (usually a single offspring per year) require high adult survival to avoid population declines (Barclay & Harder, 2003) and dictate slow recovery of impacted populations. Substances that could increase metabolic rates may affect bats that rely on

lowered metabolic rates during daily torpor by disrupting energy budgets (O'Shea & Johnson, 2009). Bats also differ from rodents and other insectivorous mammals such as shrews by physiological constraints due to hibernation and migration. Lipophilic pesticides can have a detrimental effect by accumulating in the stored fat due to the consumption of arthropods contaminated with pesticides. When fat is metabolized during hibernation or migration, the pesticide concentrations can reach high and toxic levels, especially in the brain (Clark, 1988). These life-history traits can render bat populations more susceptible to long-term effects of pesticides than other mammals and, compared to rodents, possibly more than 5 times sensitive. Therefore, even for bat species that prey only on flying insects (Table 2) a reproductive risk for bat species cannot be excluded.



First picture (L. Roos): Application of the insecticide Insegar in the study apple orchard. Second picture (L. Roos): Recording of bat activity with a batcorder installed at a height of 4.5 m at the border of the treated apple orchard.

In the performed first-tier risk assessment, it is assumed that individuals collect all their food in the treated area (worst case scenario). In reality, individuals foraging in the agricultural landscape may visit a variety of habitats within a single night and may obtain their food also in a variety of non-agricultural habitats. To calculate a refined TER, assumptions were made about the minimal time (best case scenario) an individual of a particular bat species feeds in the orchard [Appendix 6]. Following the literature *Pipistrellus pipistrellus*, *Myotis nattereri*, and *Myotis mystacinus* forage in, up to 2.4, 6, and 12 different foraging areas per night, respectively (Davidson-Watts & Jones, 2006; Dietz *et al.*, 2007). If we assume that each foraging area is used in the same proportion and, in a best case scenario, only one sprayed orchard site is used per night, 42%, 17% and 8% of the daily food intake of an individual of,

respectively, *P. pipistrellus*, *M. nattereri*, and *M. mystacinus* are likely to be contaminated with pesticides. These assumptions are speculative and radio-telemetry should be carried out in order to get more insights into bat foraging habits and to enable a more realistic risk evaluation process. However, that approach helps to place the TER values obtained under assumed best case scenarios in relation to the safety factors. For species that mainly take large part of their prey by gleaning foliage-dwelling arthropods (*M. nattereri* and *M. mystacinus*, see Table 2), the refined TER values were still below the trigger value of 5 (indicating a risk). Values for *P. pipistrellus* which may take parts of the food by gleaning ranged between 10.0 and 15.6 [Appendix 6] and thus, without having information on the sensitivity of bats to pesticides, a reproductive risk even under the assumed best-case scenarios cannot be excluded [Appendix 6].

Other orchards crops may also act as foraging areas for bats in general and, given the vegetation structure, in particular for gleaners which are especially susceptible to pesticides considering the high residues demonstrated for their prey. Davy *et al.* (2007) reported a number of bat species in an olive orchard treated with insecticides, among them the lesser horseshoe bat (*Rhinolophus hipposideros*) and Geoffroy's bat (*Myotis emarginatus*), both known as gleaner (Dietz *et al.*, 2007).

High bat activity levels were also demonstrated at several vegetable fields [Appendix 3]. Considering the massive pesticide input in these crops (Roßberg, 2007), a study of pesticide residue patterns on nocturnal arthropods is strongly suggested to get a realistic estimate for the risk of pesticide exposure. The activity of the greater mouse-eared bat, a species almost exclusively feeding on carabid beetles (Beck, 1995), was highest above the vegetable fields [Appendix 3]. Ground-dwelling arthropods such as carabid beetles may exhibit high pesticide residues especially after ground-directed applications in the afternoon. A massive die-off of juvenile greater mouse-eared bats which was attributed to the application of an organophosphate to potato fields and apple orchards in Germany (Hoffmann, 1991) already demonstrated that this species is threatened by pesticide exposure.

While in the orchards most of the airborne small insects were non-Diptera such as small moths, Diptera were the predominant group in the vegetable fields [Appendix 3; Appendix 4]. Since it has been shown that Diptera larvae can accumulate significant amounts of chemicals (Eitminavichiute *et al.*, 1982; Park *et al.*, 2009), residue patterns in vegetable fields may differ from those measured in the orchard. Research is required to examine if such an accumulation of modern and less persistent pesticides takes place in terrestrial Diptera developing in

agricultural soils, especially in vegetable fields where wet soils may increase the contact of the larvae with pesticides.

Remarkably high activity levels of all examined bat groups were detected over agricultural fields located next to forests [Appendix 3]. Given that in agricultural landscapes most forest edges are situated next to crop fields, a thorough examination of the potential pesticide exposure is necessary and special risk mitigation methods for those habitats may be required. Forest edges function as windbreaks which potentially could concentrate large densities of contaminated insects after pesticide application. The northern bat (*Eptesicus nilssonii*) and the barbastelle (*Barbastella barbastellum*) were predominantly recorded at the forest edges during the survey study [Appendix 3]. Both are rare species and a potential risk due to pesticide exposure could even have severe impacts on their populations. Research is also required to examine if Bechstein's bat and the brown long-eared bat (*Plecotus auritus*), both forest inhabiting bats exclusively taking their prey by gleaning, are using orchards situated next to forests for foraging since a high risk is expected due to demonstrated high residue values of foliage-dwelling arthropods in orchards.

5 CONCLUSION

In the course of the present thesis a quantitative bat survey methods comprising the set up of several stationary sampling systems which automatically trigger the ultrasonic recording was established. This method was suitable to survey bat activity on a landscape-scale.

The survey of bat activity and bat specific food availability revealed that vineyards, the predominant agricultural habitat in southern Palatinate, are of low quality as foraging habitats for bats due to the demonstrated low availability of small aerial insects. Furthermore it was demonstrated that the creation of artificial wetlands can benefit bats by compensating for the low availability of suitable prey insects in the surrounding vineyards. However, aerial insects with aquatic larval stages might transfer contaminants from aquatic to terrestrial systems (e.g. Walters *et al.*, 2008). To estimate the risk for bats foraging over artificial wetlands in the agricultural landscape, where pesticide inputs are common, research is required to examine if an accumulation of pesticides takes also place in aquatic insects developing there.

When comparing agricultural sites other than vineyards, forests and meadow habitats activity levels of bats species being predominately aerial hawker did not significantly differ. In several orchards and agricultural fields even higher activity levels than those recorded in the simultaneously examined meadows and forests were demonstrated. Over agricultural fields located next to forests all examined bat groups showed remarkably high activity levels.

Given this high bat activity due to the availability of suitable prey insects and the known high pesticide inputs in for example orchards and vegetable fields, it was concluded that pesticide exposure via ingestion of contaminated insects is highly likely. By measuring residues of insecticides on prey insects in an apple orchard and simultaneously demonstrating bat foraging activity, exposure of bats to pesticides was shown for the first time for European bats. By following the toxicity-exposure approach of the current European pesticide risk assessment a potential reproductive risk for bat species that at least take small parts of their prey by gleaning to pesticides was indicated. Additionally, the toxicity-exposure approach of the current European pesticide risk assessment includes a safety factor for interspecific sensitivity differences and since there are no toxicity data of modern pesticides for bats, it remains uncertain if the one applied is conservative enough. Because bats are potentially more sensitive to reproductive effects of pesticides than other mammals due to their ecological traits a reproductive risk for the other non-gleaning feeding guilds can not be excluded. My risk assessments were based on the assumptions that bats take all their food in the treated orchard (worst case) or in speculative species-specific numbers of foraging habitats based on

literature data (best case). To produce a more realistic and reliable risk assessment for bats, a research program that is investigating the sensitivity of bats to pesticides and radio telemetry studies examining to what extent agricultural habitats are individually used for foraging are required.

Based on the recorded bat activity, the reported availability of prey insects and the crop-specific pesticide regime further scenarios where pesticide use is more likely to cause a risk for bats were indicated: bats preying on soil arthropods in vegetable fields, aerial hawkers feeding on Diptera over vegetable fields, and all bat species foraging along forest edges situated next to agricultural fields. Additional studies on the pesticide contamination of the food items are necessary as a basis for realistic risk assessments of the mentioned scenarios. Moreover, bats may encounter a mixture of pesticides by foraging over a number of agricultural fields with different crops, a risk which is generally not considered so far.

The demonstrated bat diversity and activity in the agricultural landscape may also reflect an adaptation by bats to feeding in structurally less optimal habitats with high pesticide inputs as these increased in their relative availability whilst optimal habitats decreased on a landscape scale in the last decades. By far the most commonly recorded species in our study was the common pipistrelle which was in accordance with other studies (e.g. Lisón & Calvo, 2011). *Pipistrellus pipistrellus* is a habitat generalist (Dietz *et al.*, 2007) which makes them less vulnerable to differences in prey diversity and structural landscape changes caused by agricultural intensification. In some cases it has been suggested that the expansion of *P. pipistrellus* populations could contribute to the decline of threatened bat species such as the lesser horseshoe bat *Rhinolophus hipposideros* (Arlettaz *et al.*, 2000; Lisón & Calvo, 2011), a species whose massive and large-scale population declines are often related to the past use of pesticides (e.g. Bontadina *et al.*, 2000; Bontadina *et al.*, 2008). Therefore it is very likely that the bats that we observe today in the agricultural landscape are the species that are least sensitive to pesticides.

These faunal changes and species impoverishments as a result of the agricultural intensification cannot be reversed by only minimizing the risk of pesticides due to the implementation of bats in the pesticide risk assessment approach. In addition it also requires a landscape scale management approach with a focus on the creation of suitable foraging habitats for bats such as woodland edges and wetlands. Together with an enhanced risk assessment of pesticides these measurements could increase bat density and diversity in the agricultural landscape and also bring the ecosystem service provided by this group, pest suppression, to its full potential.

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APPENDICES

Appendix I Bats as bioindicators – the need of a standardized method for acoustic bat activity surveys

Bats as bioindicators – the need of a standardized method for acoustic bat activity surveys

Peter Stahlschmidt & Carsten A. Brühl (2012)

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Bats as bioindicators – the need of a standardized method for acoustic bat activity surveys

Peter Stahlschmidt* and Carsten A. Brühl

Institute for Environmental Sciences, University of Koblenz-Landau, Fortstr. 7, 76829 Landau, Germany

Summary

1. The species-rich group of bats fills a wide range of ecological niches and provides ecosystem services like pest control. Bats are known to be sensitive to environmental stressors and could, therefore, be used in assessing ecosystem quality. To use bats as bioindicators, a standardized bat survey method needs to be established as the existing approaches vary in their methodology, and results are, therefore, often not comparable.
2. Generally, there are two different acoustic bat survey methods: the transect walk and the stationary measurement. By conducting transect surveys and simultaneously using several stationary systems, we measured bat activity within a homogeneous habitat and evaluated which method assessed the spatial bat activity patterns with highest precision. Also the survey tool – the detectors themselves – can be grouped into devices with two different methods of triggering the recording of ultrasonic signals: actively by a fieldworker or automatically by a built-in recording control algorithm of the detector. We measured bat activity simultaneously and side by side with both methods for direct comparison.
3. Our results indicate that the transect survey fails to represent the heterogeneous bat activity patterns in a homogeneous landscape. Furthermore, errors occur based on the subjective hearing of the active triggering of the data recording by the human operator.
4. The application of several stationary and automatic sampling systems has the highest potential for standardized acoustic bat surveys. The general use of such an approach would enable us to understand bat activity at landscape scale and could lead to an improvement of bats as bioindicators.

Key-words: acoustic method, Anabat, detector, ecoObs-batcorder, heterodyne system, monitoring, Pettersson D240X, Pettersson D500X, Song Meter SM2BAT, transect survey

Introduction

Recently, Jones *et al.* (2009) argued that bats have great potential as bioindicators. Changes in bat populations or activity were related to climate change, water quality, agricultural intensification, loss and fragmentation of forest habitats, and habitat pollution (Jones *et al.* 2009).

Most current bat surveys rely on acoustic methods. Contrary to capture methods, telemetry and direct observations, the use of ultrasonic detectors is often the only logistically feasible and cost-effective survey method. However, fundamental concerns regarding the basic methodological designs of many acoustic surveys were expressed as they often fail to address temporal and spatial variation in bat activity patterns (Hayes 2000; Sherwin, Gannon & Haymond 2000; Gannon, Sherwin

& Haymond 2003). The assessment of variation in bat activity pattern and the comparability of independent studies are basic requirements for the potential use of bats as bioindicators. Consequently, a standardized bat survey method using a suitable detector system has to be established.

In practice, there are two different methods to survey bat activity: (i) line transects and (ii) the stationary measurement. The transect method is based on the assumption that the bat activity recorded along a transect is in accordance with the activity in the whole habitat of concern (i.e. the habitat is supposed to be homogeneous in regard to bat activity). In contrast, stationary systems are set up at a selected site to reflect the overall activity of the corresponding habitat. To test the transect survey assumption of evenly distributed bat activity within a habitat, we measured bat activity in an agricultural landscape dominated by cereal fields, a habitat assumed to be completely homogeneous, by conducting a transect survey while simultaneously recording with several stationary systems.

*Correspondence author. E-mail: stahlschmidt@uni-landau.de

In addition to the two different survey methods, there are also two different methods of triggering the recording of ultrasonic signals: (i) actively by a fieldworker using heterodyne or frequency division systems [e.g. Pettersson D200 and D240X (Pettersson Electronic AB, Uppsala, Sweden) or S-25 bat detector (Ultra Sound Advice, London, UK)] or (ii) automatically by the detector devices [e.g. Anabat II (Titley Electronics, Ballina, Australia) or ecoObs-batcorder (ecoObs GmbH, Nürnberg, Germany)]. We measured bat activity simultaneously and side by side with a hand-held Pettersson D240X detector in the heterodyne modus and an automatic ecoObs-batcorder in open landscapes and forests to examine whether differences in data recording occur because of the subjective hearing of the operator of the active-triggering system in comparison with a built-in recording control algorithm of an automatic device.

Material and methods

The fieldwork was conducted around Landau (S-Germany). To avoid seasonal differences, bat activity was recorded from June to August (2008–2009). All recordings were obtained during nights with temperatures between 16 and 21 °C, wind speed below 10 km h⁻¹ and without rain.

ADDRESSING SPATIAL VARIABILITY OF BAT ACTIVITY – TRANSECT WALKS VS. MULTIPLE STATIONARY MEASUREMENTS

We conducted transect surveys along a 4-km loop trail (Fig. 1) in a homogeneous agricultural landscape consisting mainly of cereal fields. We chose an area free from linear features like woodland edges or hedgerows as bats use them as flight paths (Verboom & Huitema 1997). Bat activity on the loop trail was detected with the heterodyne system of a Pettersson D240X by continuously scanning between 20 and 60 kHz to cover the frequency ranges of expected bat species. High input gain of the detector was selected, and headphones were

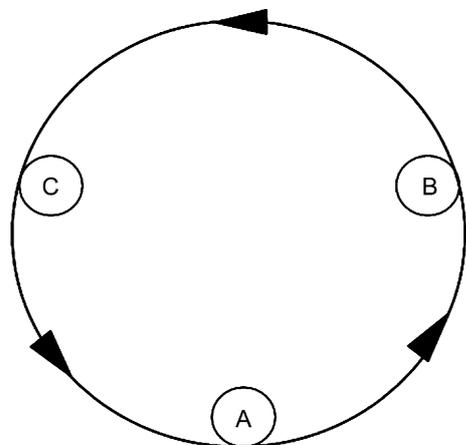


Fig. 1. Schematic diagram of the survey design. Transect surveys were performed along a 4-km loop trail (c. 1 h). Simultaneously, bat activity was recorded at three stationary sampling points (A, B, C) by the means of ecoObs-batcorders which were installed at a height of 250 cm at equal distances (c. 1.3 km) to each other along the loop trail.

used to avoid interfering background noise. The first author of this study walked at a constant speed (c. 1 h for the transect) holding the Pettersson detector at a 45° direction relative to the ground and at a height of about 170 cm. Starting 1 h after sunset, the same transect was walked during nine nights from July to August 2009. Simultaneously, three ecoObs-batcorders were installed at a height of 250 cm at equal distances (c. 1.3 km) to each other along the loop trail as stationary sampling points (Fig. 1: referred to as sites A–C). The threshold influencing the recording range was set to a fixed sensitivity (full gain at 40 kHz and 96 dB SPL), which resembles a recording radius of about 10 m for most European bat species (Runkel 2008). Calls were determined using sonograms produced with the software bcAnalyze version 1.10 (ecoObs GmbH). *Pipistrellus pipistrellus* was the only occurring bat species. A pass was defined as a sequence of calls that end five or more seconds before the next sequence begins. The differences in numbers of recorded *P. pipistrellus* passes were analysed using paired *t*-tests for each combination of the stationary sampling sites. The numbers of bat passes were normally distributed.

COMPARISON OF SIMULTANEOUS ACTIVE AND AUTOMATIC TRIGGERING OF ACOUSTIC BAT RECORDING

In this approach, bat activity was measured along small forest paths (referred to as forest habitats; $n = 12$) and homogeneous agricultural areas without any linear elements (referred to as open landscape; $n = 27$). At each location, bats were detected for 1 h per night simultaneously by an ecoObs-batcorder placed on top of a 170-cm pole (automatic approach) and by a Pettersson D240X held at a height of 170 cm and at a 45° direction relative to the ground (active approach). The sampling points at the forest path were chosen to have at least a radius of 10 m of uncluttered acoustic space around. The ecoObs-batcorder and the Pettersson detector were used as described previously. In the study area, only *P. pipistrellus*, *Nyctalus noctula*, *Nyctalus leisleri* and *Eptesicus serotinus* were regularly present. Because of the overlapping between acoustic repertoires of the latter three species, it was impossible to assign all call sequences to one of those species with sufficient confidence. Hence, the three species were assigned to the group *Nyctalus–Eptesicus*. This resulted in two different acoustic groups: *P. pipistrellus* calling at the upper end of the recordable frequency scale (35–55 kHz) and *Nyctalus–Eptesicus* calling at lower frequencies (20–35 kHz). The differences in the number of bat passes between both approaches were analysed using paired *t*-tests for each group of bats and for each habitat type. A bat pass was defined as stated earlier. The numbers of passes were normally distributed.

Results

ADDRESSING SPATIAL VARIABILITY OF BAT ACTIVITY – TRANSECT WALKS VS. MULTIPLE STATIONARY MEASUREMENTS

Site A revealed on average 3–5 times the number of *P. pipistrellus* passes than sites B and C (Table 1A, Fig. 2). Paired *t*-tests between all three possible combinations of the stationary recording sites in number of detected *P. pipistrellus* passes showed significant differences in the numbers of passes at the three sites (Table 1B).

Table 1. Spatial variation of bat activity in a homogeneous landscape. (A) Number of recorded *Pipistrellus pipistrellus* bat passes at three different stationary sites and on a transect walk in a homogeneous agricultural landscape. Bat activity was measured for 1 h at nine different days between July and August 2009. (B) Statistical differences of *P. pipistrellus* bat passes between all combinations of the three stationary recording sites. *P*-values derived from paired *t*-tests

	<i>n</i>	Mean ± SD (minimum–maximum)		
(A)				
Site A	9	16.7 ± 0.7 (14–19)		
Site B	9	3.2 ± 0.4 (2–5)		
Site C	9	5.4 ± 0.6 (3–8)		
Transect	9	4.7 ± 2.4 (0–17)		
	d.f.	<i>t</i> -value		<i>P</i>
(B)				
Site A/Site B	8	19.5		< 0.001
Site A/Site C	8	14.4		< 0.001
Site B/Site C	8	–4.8		0.001

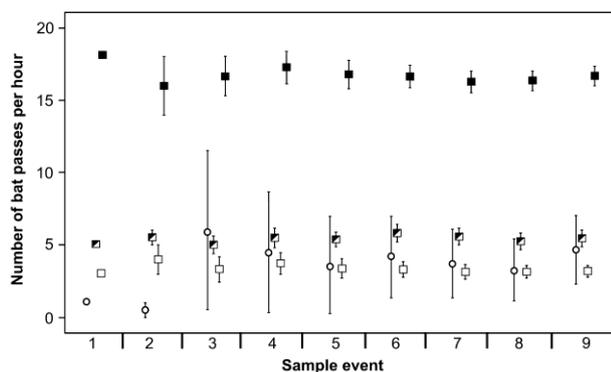


Fig. 2. Mean and standard error (bars) of recorded *Pipistrellus pipistrellus* passes per hour in a homogeneous agriculture landscape in dependence of the number of sample events (sampling in chronological order). Passes were recorded by a Pettersson D240X during a transect survey (○) and by three stationary sampling points along the transect by the means of ecoObs-batcorders (Site A: ■; Site B: □; Site C: ●).

Compared with the data of the three stationary devices, detecting of bat activity by walking transects resulted in less precise measures of activity as seen by the large standard errors around the mean value of the number of recorded *P. pipistrellus* passes (Table 1A; Fig. 2).

COMPARISON OF SIMULTANEOUS ACTIVE AND AUTOMATIC TRIGGERING OF ACOUSTIC BAT RECORDING

For passes of *Nyctalus–Eptesicus*, the simultaneously acquired data sets of both approaches were significantly different, with the active approach always detecting more passes. The differ-

ence was more pronounced in the open landscape ($t = -6.24$, d.f. = 26, $P < 0.001$; Table 2) than in the forest habitats ($t = -2.55$, d.f. = 11, $P = 0.027$; Table 2). On average, the active approach detected 3.6 times more *Nyctalus–Eptesicus* passes in the open landscape and 1.4 times more *Nyctalus–Eptesicus* passes in forests in comparison with the automatic ecoObs-batcorder (automatic approach). For *P. pipistrellus*, the active approach detected significantly more bat passes only in the open landscape ($t = -3.22$; d.f. = 26, $P = 0.003$; Table 2), on average 1.1 times more than the ecoObs-batcorder. No statistically significant difference was detected between both methods in the forest habitats ($t = -1.15$; d.f. = 11, $P = 0.275$; Table 2).

Discussion

SAMPLING METHOD FOR STANDARDIZED BAT ACTIVITY SURVEYS

Patterns of bat activity may vary within a habitat in response to a variety of biotic (e.g. abundance of prey insects) and abiotic (e.g. landscape structure) factors. Our results demonstrated significantly different bat activity patterns even in a homogeneous agricultural landscape. We assume that spatial heterogeneity of bat activity is much more pronounced in habitats with higher structural heterogeneity such as forests (within a forest there are clearings, young undergrowth, mature trees). Measurements from transect surveys give information about bat activity on the landscape scale, but fail to account for spatial variation and, therefore, may miss small but important foraging areas that could result in inappropriate management recommendations. Moreover, the transect method cannot assess spatial and temporal activity at a given site and, therefore, may miss vital periods of bats at certain parts of the transect. The stationary and simultaneous measurement at a number of detecting sites within a habitat accounts for this spatial and temporal variation of bat activity and is, therefore, the recommended approach for a standardized bat activity survey.

SAMPLING DEVICE FOR STANDARDIZED BAT ACTIVITY SURVEYS

In acoustic surveys, bat activity cannot be compared across species (Jones, Vaughan & Parsons 2000) because of species-specific differences in frequency ranges and intensity of echolocation that are subject to different levels of frequency-dependent atmospheric attenuation (Lawrence & Simmons 1982). For example, the longer calls, the lower amplitude and the less rapid attenuation of the lower frequency calls of *Nyctalus–Eptesicus* result in an enlarged detection range of that group compared with *P. pipistrellus*. Nevertheless, the comparison of activity is possible for each species independently as long as the detection capability is constant across all habitats. We compared simultaneously performed active and automatic triggering of the detecting of two acoustically different bat groups in two habitats that differ in the amount of acoustic clutter and demonstrated different detection ratios of both

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Table 2. Statistical comparison of the number of bat passes detected by active and automatic triggering of acoustic recording in dependence of the clutterness of the habitat. Bat passes (*Nyctalus-Eptesicus* and *Pipistrellus pipistrellus*) were detected side by side and simultaneously by a fieldworker using a Pettersson D240X (active approach) and an ecoObs-batcorder (automatic approach) at several sites in the open landscape and forests for 1 h. The ratio between the means of the active approach to the automatic approach is given. *P*-values derived from paired *t*-tests

	Mean ± SD (recorded bat passes)					
	Active approach	Automatic approach	Ratio	d.f.	<i>t</i> -value	<i>P</i>
Open landscape						
<i>Nyctalus-Eptesicus</i>	6.9 ± 4.7	1.9 ± 0.9	3.6	26	-6.24	< 0.001
<i>P. pipistrellus</i>	8.7 ± 7.1	8.0 ± 6.7	1.1	26	-3.22	0.003
Forest						
<i>Nyctalus-Eptesicus</i>	2.2 ± 0.9	1.6 ± 0.7	1.4	11	-2.55	0.027
<i>P. pipistrellus</i>	6.1 ± 2.3	5.9 ± 2.5	1.0	11	-1.15	0.275

groups depending on the habitat. Systems with automatic triggering of the acoustic detection such as the ecoObs-batcorder have a definite and standardized detection amplitude threshold (Runkel 2008), whereas the human operator, by using a heterodyne system, can detect 'expected' signals even within the background noise. Thus, compared with the forest where higher level of clutter leads to scattering, spreading, absorption and reflection of the echolocation calls (Griffin 1971; Parsons 1996), in the open landscape, low-frequency calls arriving from a greater distance can be detected by the fieldworker (active approach) quite well. This expanded detection range of the active approach results in an overestimation of the activity of bat species using low frequency calls (e.g. *Nyctalus-Eptesicus*) in uncluttered habitats such as the open landscape. This bias of the active approach violates the assumption that the amount of bat calls of a given species detected at a site reflects the intensity of habitat use, and comparisons between different habitats are, therefore, misleading. In addition, the active-triggering approach depends on the skills, the subjective hearing ability, concentration or tiredness of the operator in the field leading to further potential biases.

Active triggering is labour-intensive, as it requires one person per sampling site and detector, whereas several automatic recording units can be handled by a single person alone. In the active approach, it is, therefore, common practice that bat activity is only measured during a certain nocturnal time span (e.g. Kusch *et al.* 2004). But temporal activity patterns of bats

may considerably vary on a nightly basis in response to a variety of factors including abundance of prey insects and weather conditions (Hayes 1997; O'Donnell 2000). Moreover, the output signals of heterodyne systems as used in active-triggering approaches contain no information on the duration and frequency of the original input signal and are, therefore, unsuitable for further bioacoustic analysis.

In contrast, automatic triggering seems to be a promising tool for an unbiased and comparable assessment of bat activity (see Table 3 for a comparison with the active approach). However, the range of automatically triggered recording devices available differs in the extent to which they are suitable for a standardized bat activity survey. Simple automatic systems for recording of bat activity can be assembled by connecting a voice-activated tape recorder to a heterodyne detector (e.g. O'Donnell 2000) or a time-expansion detector, respectively. In the heterodyne system, a frequency dial has to be set, limiting this approach to the detection of bat calls within the selected narrow frequency range (Parsons & Szewczak 2009). During the output phase of the time-expansion system, it is not possible to record further bat calls (Parsons & Szewczak 2009). Thus, both types of self-assembled automatic systems are unsuitable for a standardized bat survey method.

The remaining automatic detector systems can be divided into zero-crossings period meters (Anabat II) and recently developed detectors systems with high-speed analogue to digital converters that can directly record ultrasound (real time

	Active	Automatic	Automatic
Triggering of the detection	Active	Automatic	Automatic
Transforming of the ultrasound prior to recording	Heterodyning	Zero-crossing	Direct recording
Examples	Pettersson D200 S-25 detector	Anabat II	ecoObs-batcorder Pettersson D500X SM2BAT
Labour intensity	High	Low	Low
Quality of data	Low	Limited	High
Comparability of the results	Not possible (subjective biases)	Not possible (directionality)	Possible*
Purchase price	c. 300 €	c. 2000 €	800–3000 €

Table 3. Overview of the different commercially available approaches for the stationary measurement of bat activity in regard to their labour intensity, quality of the acoustic data, comparability of results and purchase prize. Self-assembled automatic systems are not considered

*If used with exposed microphone and calibrated (only the ecoObs-batcorder fulfils that in its standard version).

recorder) such as the ecoObs-batcorder, the Pettersson D500X (Pettersson Electronic AB) and the Song Meter SM2BAT (Wildlife Acoustics; Concord, USA). We discuss the suitability of these detector systems to meet the demands of a standardized stationary bat survey in the following (see Table 3 for an overview).

Quality of the recording data

Analysis of zero-crossing period meters shows only information about the strongest harmonic in any signal (Fenton *et al.* 2001), but fine spectrotemporal details are missing. This reduction can complicate species determination within some genera (Fenton 2000; Parsons, Boonman & Obrist 2000; Parsons & Szwczak 2009). Ultrasound can be recorded directly with real-time recorders, and untransformed and uncompressed data files such as WAV files are produced. The preservation of all characteristics of the original signal allows detailed bioacoustical descriptions of bat calls (Jones, Vaughan & Parsons 2000; Parsons & Szwczak 2009).

By considering the vast amount of call sequences one can get with several autonomous recording units, the manual measurement of call characteristics becomes a bottleneck. To cope with this problem, multivariate identification software can be applied (Jones, Vaughan & Parsons 2000; Parsons & Szwczak 2009). These identification systems rely on the high-quality data of real-time recording (Parsons & Szwczak 2009). Apart from saving time and removing any subjectivity from species identification (Jones, Vaughan & Parsons 2000), a further advantage of identification systems is that they can also be used by nonspecialists in acoustics.

Recent studies use the feeding buzz rate of bats as an indicator of the value of particular areas as foraging habitats (e.g. Vaughan, Jones & Harris 1997). A feeding or final buzz is in general a sequence of calls becoming shorter in duration and broader in bandwidth and is emitted by aerial feeding bats during prey-capture attempts (Griffin, Webster & Michael 1960). Feeding buzzes are especially faint and difficult to record with the Anabat II (Scott *et al.* 2010) compared with detectors that use direct recording as only strong portions of any call will activate the zero-crossing period meter (Fenton *et al.* 2001).

Comparability of results

To compare results obtained by more than one recording unit, detection fields of identical size and shape are required.

Individual units of the same detector system can vary extensively in the size of their detection fields (Waters & Walsh 1994; Fenton 2000; Larson & Hayes 2000). The units can be calibrated against each other by using an external ultrasonic sound (e.g. Larson & Hayes 2000 for the Anabat II system), but fine-scale adjustments require a signal generator in a controlled laboratory environment. The ecoObs-batcorder is so far the only commercially available detector system that is calibrated in a standardized way (Runkel 2008) and, therefore, allowing direct comparisons of independent studies.

The shape of the detection field depends on the directionality of the microphone. The directionality itself is a function of the microphone type and its position. Condenser microphones and recessed microphone positions increase directionality, while miniature electret microphones and exposed microphone positions allow greater omni-directionality (Pye 1993; Waters & Walsh 1994; Runkel 2008). Because of the condenser microphone and its fixed position on the detector surface, the Anabat II shows high directionality, resulting in a detection field being longer than wide (Larson & Hayes 2000). This can lead to differences in the number of recordings at the same site because of the orientation of the detector and may result in biases between sites (Weller & Zabel 2002). The Pettersson D500X, SM2BAT and ecoObs-batcorder have omni-directional miniature electret microphones. While there are options to use an external microphone extended on a cable in the Pettersson D500X and SM2BAT system, the ecoObs-batcorder already has an external microphone in an exposed position in its standard design.

Conclusion

Considering our results, the use of several randomly selected stationary sampling points within a habitat using calibrated and automatically triggered real-time recording devices (e.g. ecoObs-batcorder) has the highest potential for standardized acoustic bat surveys. The proposed approach produces unbiased and comparable data sets on the relative activity of bats. In addition, the use of stationary and automatic recording systems is less labour-intensive and time-consuming and even feasible for nonbat-specialists and, therefore, represents a cost-effective survey method.

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Appendix II The potential of a combined acoustic detection field to explore flight patterns of bats

**The potential of a combined acoustic detection field to explore
flight patterns of bats**

Peter Stahlschmidt & Carsten A. Brühl

Manuscript

The potential of a combined acoustic detection field to explore flight patterns of bats

Peter Stahlschmidt * & Carsten A. Brühl

Institute for Environmental Sciences, University of Koblenz-Landau, Fortstr. 7,
76829 Landau, Germany

*Correspondence author. E-mail: stahlschmidt@uni-landau.de

ABSTRACT

Recordings of a single bat-detector do not allow any conclusion if the recorded bat activity at a particular site is caused by foraging or commuting bats. Moreover, no information on the number of individuals involved in that recorded activity can be given. We tested the potential of a combined detection field of multiple, adjacent and simultaneously recording stationary bat-detectors (batcorder) and demonstrated its potential to get insights in flight pattern and a better estimation of the number of individuals on which the activity measurements are based on. This approach is a useful tool to get more detailed information on the importance of small scaled habitats such as clearings for bat foraging. Moreover, the proposed combined detection field has the potential to reveal transfer paths of commuting bats.

INTRODUCTION

In recent years there has been increased interest in the study of bat activity (Jones *et al.*, 2009). Depending on the research requirements a variety of methods has been employed. Telemetry is used to study the activity of individuals. Information on site specific bat activity can be obtained by direct observations and echolocation surveys. Direct capture by the means of nets is used to assess and identify flying bats at a particular site.

However, all these methods fail if the research requires statements whether reported bat activity at a particular site is caused by foraging or commuting bats. It is common practice to estimate the quality of a site as a foraging habitat by recording bat activity and quantifying the feeding buzzes (e.g. Russo and Jones, 2003). However, some bat species do not produce feeding buzzes (Fenton and Bell, 1979) and bats may also hunt while commuting along transfer paths if appropriate insects are available. Furthermore, acoustic surveys cannot be used to quantify number of bats in an area as it is not possible to distinguish between a single individual of a species passing the detection field of a detector several times and several individuals each passing it once (Hayes, 2000).

We tested if combined and overlapping detection fields of several simultaneously recording stationary bat detectors have the potential to get insights in individual flight patterns and allow conclusions about the number of flying individuals. We tested this approach at two sites, one of them being a habitat patch enclosed by tree rows and assumed to be used by bats for foraging and the other one being a field track in an agricultural landscape assumed to be used as a part of a transfer path.

MATERIALS AND METHODS

The field work was conducted in 2008 and 2009 in the southern part of Rhineland-Palatinate (Germany) around Landau. We recorded bat activity using automatic stationary bat detector systems, so-called batcorders (ecoObs GmbH, Nürnberg, Germany). A batcorder is assumed to have a detection radius of approximately 10 m (Runkel, 2008).

We generated combined detection fields at two different sites which were both known for high bat activity due to previously performed activity surveys with a handheld Pettersson D240X bat detector (Pettersson Electronic AB, Uppsala, Sweden). The first site (site 1) was a

grassy area of approximately 10 m x 110 m adjacent to an apple orchard and surrounded by apple tree rows at the far three sides (Fig 1a). Batcorders were positioned in a 2 by 7 grid (Fig. 1a) with a distance of 15 m between adjacent batcorders. All batcorders were mounted on steel rods at a height of 450 cm. As a result a detection field of approximately 35 x 110 m was generated (Fig. 2) covering the grassy area completely as well as a part of the apple orchard. The second site (site 2) was situated at an approximately 5 m wide track between vineyards. The detection field of site B (approx. 50 x 50 m), generated by 9 batcorders equally spaced at 15 m in a 3 by 3 grid at a height of 450 cm comprised the track and parts of the adjacent vineyard (Fig. 3a).

The batcorders were adjusted to the standard settings (Runkel, 2008; Stahlschmidt and Brühl, 2012). The post trigger, defined as the interval between two successive detected calls that are written into the same sound file, was set to 600 ms. The critical frequency was adjusted to 16 kHz to eliminate lower frequency signals in the call recognition algorithm. Prior to recording, clocks of the batcorders were synchronized. Species were determined using sonagrams produced with the software bcAnalyze version 1.10 (ecoObs GmbH, Nürnberg, Germany). Flight patterns were detected by comparing the recordings of all detectors of the combined detection fields.

RESULTS

Fig. 1.b shows a time interval (80 seconds) of representative chronological recordings of *Pipistrellus pipistrellus* (Schreber, 1774) of the 14 simultaneously recording batcorders of site 1. Gaps in the sequence of recorded calls are caused by bats leaving the detection field for a short time. The sequences of the present recordings demonstrated that they were caused by two individuals. The first individual spent 80 seconds in the detection field and made several turns. The second individual entered the detection field in the 39th second and left it after 8 seconds. When considering each batcorder separately and following the method of Fenton (1970) by defining one call sequence with not more than one second between sequential call sequences as a bat pass, one has to interpret the recordings of the shown time interval as up to eight bat passes from different individuals (when only considering batcorder B; Fig. 1b). By comparing the recordings of all batcorders the approach revealed flight patterns and it became clear that there were only two bat individuals flying in the depicted time interval and that all call sequences recorded by batcorder B were caused by a single individual (Fig. 1 b).

A representative chronological recording of 9 simultaneously recording batcorders from site 2 is shown in Fig. 3.b. In the presented time interval of 80 seconds three *P. pipistrellus* (probably 3 different individuals) entered the detection field and left it after 3-4 seconds. The three recorded individuals were flying in the same direction, only passing through the detection field covering the trail and without making any turns. No bat passes were recorded by the batcorders situated over the vineyards.

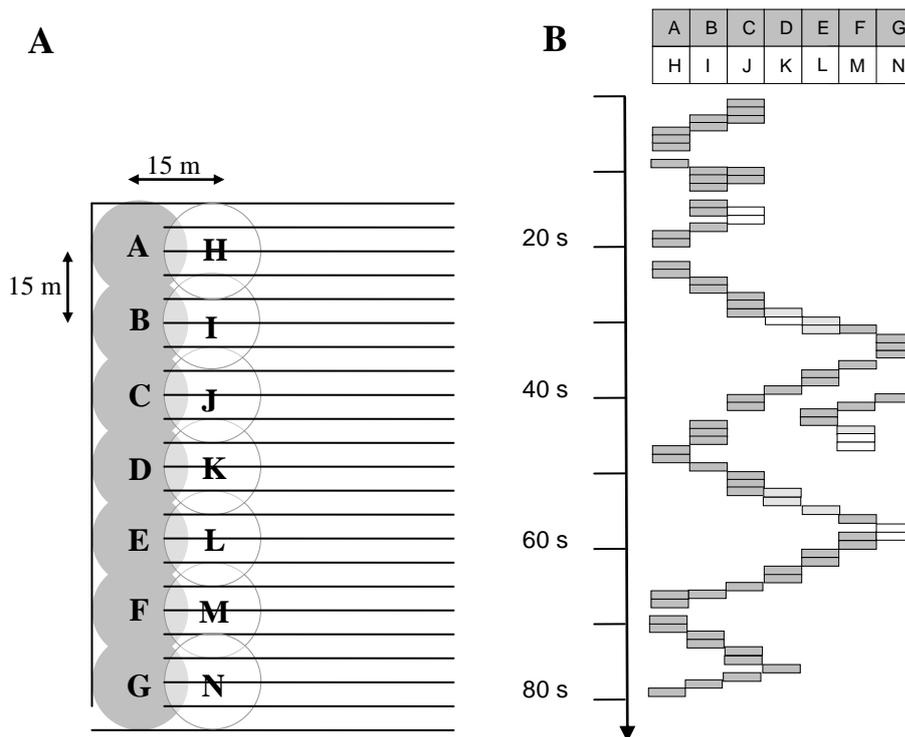


Fig. 1. A. Schematic diagram of site 1 (lawn area adjacent to an apple orchard). Apple tree rows are indicated by lines, the positions of the batcorders by numbers (A-N), and the recording areas of the batcorders by circles (based on a recording radius of 10 m). **B.** Representative chronological recording runs of *Pipistrellus pipistrellus* call sequences of 14 simultaneously recording batcorders (A-N) during a time interval of 80 seconds. Each box indicates that the corresponding batcorder recorded at least one call in the corresponding time interval of one second. Dark grey coloured boxes belong to the corresponding batcorder of the first row (A-G), white coloured boxes to the second row (H-N). Light grey coloured boxes indicate that bat calls were recorded by a batcorder of the first row and the respective batcorder of the second row.

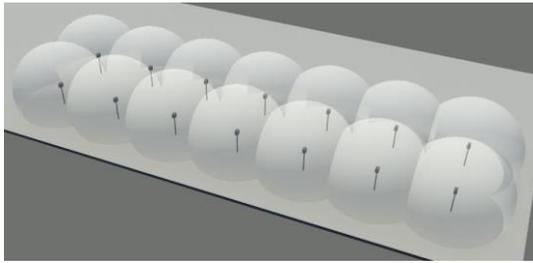


Fig. 2. Combined detection field of 14 simultaneously recording batcorders of site 1. The distance between both rows and each batcorder within the rows amount for 15 m. Batcorders were placed on top of 450 cm poles.

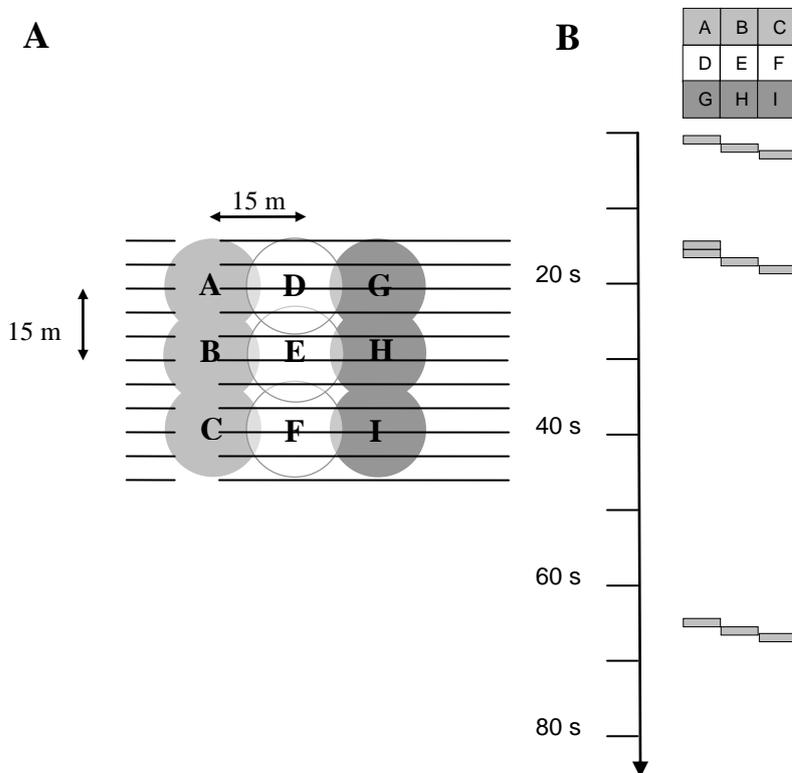


Fig. 3. A. Schematic diagram of site 2 (field track between vineyards). Vineyard rows are indicated by lines, the positions of the batcorders by numbers (A-I), the recording areas of the batcorders by circles (based on a recording radius of 10 m). **B.** Representative chronological recording runs of *Pipistrellus pipistrellus* call sequences of 9 simultaneously recording batcorders (A-I) during a time interval of 80 seconds. Each box indicates that the corresponding batcorder recorded at least one call in the corresponding time interval of one second. Light grey coloured boxes belong to the corresponding batcorder of the first row (A-C), white coloured boxes to the second row (D-F) (not recorded), and dark grey coloured boxes to the third row (G-I) (not recorded).

DISCUSSION

Obtaining information on the behaviour of flying bats at a given site is limited by the available methods. Observations with naked eyes (e.g. Ahlén and Baagøe, 1999) are only effective during dusk and dawn. The use of thermal infrared cameras (Stahlschmidt *et al.*, 2012) provides high quality information on the behaviour of individuals but the observation area is very constricted by its limited field of vision. Radar can also be used to quantify presence of bats and their flight directions (Gauthreaux *et al.*, 2008; Hayes *et al.*, 2009). However, key limitations of this technique are the inability to distinguish birds from bats and among species of bats (Larkin, 2005) as well as the difficulty to detect bats flying close or within the vegetation (Hammer *et al.*, 1995; Hayes *et al.*, 2009).

With acoustic detectors information on site specific activity can be obtained, but with this method it is not possible to distinguish between foraging and commuting bats. Furthermore, the recording of several subsequent passes of the same bat species does not allow any conclusions about the number of involved individuals. Therefore, all recorded call sequences have to be interpreted as passes of potentially different bat individuals. In the present study, however, we have demonstrated that comparing the recordings of all detectors of a combined detection field revealed flight patterns which also allow better estimates of the number of flying bat individuals at a given time period. By using a single detector and considering the number of passes as an activity measurement, the concentrated bat activity in small habitats, compared to more diffuse activity in large homogeneous habitats, could lead to an overestimation of bat activity (Celuch and Zahn, 2008). By considering bat passes as connected recording sequences of several detectors of a combined detection field a more profound comparison between different sites in regards of the number of foraging bat individuals is possible.

The multiple turns in the recorded flight pattern at site 1 of the present study made clear that the present bats were foraging. The approach of a combined detection field is a useful tool to examine if previously recorded bat activity in small scaled habitats such as clearings or limited areas enclosed by trees or hedgerows (e.g. gardens) are used by bats for foraging. A similar approach with a combination of horizontally and vertically installed detectors would be able to provide information on three-dimensional stratification of foraging activity in e.g. forests, clearings or along forest paths. In contrast, recordings of a short time interval of site 2 revealed three bat passes that always started and ended at the same detection sites located over the field track while no activity was recorded by the batcorders situated over the vineyards.

This flight pattern demonstrated that the bats used the track as a flight path. In applied ecology (e.g. ecological impact assessment in road-construction) it could be important to know if a certain area is part of a transfer path of bats. As demonstrated, the examination of flight patterns recorded by detectors of a combined detection field erected along a potential transfer path (e.g. linear landscape elements) allows the differentiation between foraging and commuting activity as well as the identification of the flight direction.

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Appendix III Bat activity in the agricultural landscape – the ultrasonic silence?

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Peter Stahlschmidt, Melanie Hahn & Carsten A. Brühl

Manuscript

Bat activity in the agricultural landscape – the ultrasonic silence?

Peter Stahlschmidt*, Melanie Hahn & Carsten A. Brühl

Institute for Environmental Sciences, University of Koblenz-Landau, Fortstr. 7,
76829 Landau, Germany

*Correspondence author. E-mail: stahlschmidt@uni-landau.de

ABSTRACT

Although agriculture dominates much of Europe's landscape, there is virtually no information on how bats use different crops for foraging. Consequently, little is known about the effects of pesticide exposure on bats and there are currently no specific regulatory requirements to include bats in European Union pesticide risk assessments for the registration of these chemicals although other mammals are considered. To evaluate the potential pesticide exposure of bats, we studied bat diversity and activity as well as the availability of aerial prey insects in different crops and semi-natural habitats in south-western Germany in a landscape dominated by agriculture. In 300 sampling nights more than 24,000 bat call sequences were acoustically recorded and, in parallel, almost 110,000 insects of suitable prey sizes were sampled by light traps. A total of 14 bat species were recorded, among them the locally rare and critically endangered northern bat (*Eptesicus nilssonii*) and the barbastelle (*Barbastella barbastellum*), all of them also occurring over agricultural fields. In comparison to agricultural habitats, higher activity levels in forest sites were only found for *Myotis* species but not for species of the genera *Pipistrellus*, *Eptesicus* and *Nyctalus*. There were no significant differences in the availability of aerial nocturnal insects between forest, meadow and agricultural habitats. Comparing the different agricultural crops, significantly fewer bat call sequences and less nocturnal insects were collected above the vineyards compared to orchards, cereal and vegetable fields. Remarkably high activity levels of all bat species were recorded above agricultural fields situated next to forests. Given the high bat activity levels recorded at several agricultural sites, among them orchard and vegetable fields both known for their high pesticide inputs, and the availability of suitable prey insects, we conclude that a pesticide exposure via ingestion of contaminated insects is likely. This potential risk is currently not considered in the European pesticide risk assessment scheme.

INTRODUCTION

Rachel Carson's (1963) classic book *Silent Spring* has immortalized the detrimental effects of organochlorine pesticides on the environment in general and on birds in particular. In the 1960s and 1970s it was also demonstrated that these pesticides were responsible for significant mortality of some bat populations in Europe and the USA (e.g. Jefferies, 1972; Gelusco et al. 1976; Clark et al., 1978). The offending highly toxic and persistent pesticides have been replaced by modern pesticides in the European Union and many other countries in the 1970s and 1980s. In the recent decades, however, applications of pesticides and chemical fertilizers have even increased and, simultaneously, the agricultural landscape heterogeneity has been greatly reduced (Benton et al., 2003). Both aspects of agricultural intensification have been associated with new losses in biodiversity and are sometimes indicated as the *Second Silent Spring* (e.g. Krebs et al., 1999). So far, little is known about the relative contribution of habitat loss and use of chemicals to the negative effects on biodiversity. Recently, Geiger et al. (2010) examined the impacts of several factors of agricultural intensification and identified the use of pesticides to have the most consistent negative effects on species diversity.

The need for assessing the risk of pesticide exposure on non-target organisms is recognized by the regulatory agencies such as the European Food and Safety Authority (EFSA). No authorisation is granted for new pesticides unless a risk assessment demonstrates that no risk for wildlife species occurs when the pesticide is applied under field conditions (EFSA, 2009). The current procedure also includes a risk assessment on birds and mammals (EFSA, 2009). There, insectivorous mammals are represented only by shrews but no reference is made to bats, although they are still reported as being threatened by pesticides (e.g., O'Shea & Johnson, 2009) and comprise one-fifth off all European mammals with a very specific ecology. The reason for this omission is probably related to the scarcity of ecological data and limited knowledge about the occurrence and activity of bats in agricultural crops.

To estimate the pesticide exposure of bats we need to know which species occur in which crop and to what extent. In this study we therefore recorded bat activity and availability of nocturnal prey insects in a multitude of agricultural sites and compared the recorded activity levels to activity levels recorded simultaneously in nearby habitats known to be used for foraging such as forests and meadows. Furthermore, we examined if recorded bat activity in

the agricultural landscape is related to the habitat type (i.a. forest, forest edge and open landscape), the crop, and the nocturnal insect abundance.

MATERIALS AND METHODS

Study sites and sampling

The study was conducted in an agricultural landscape in Rhineland-Palatinate, SW Germany around Landau. The climate of the region is characterised by an average annual temperature of 10°C and a precipitation of about 600-800 mm. The sample sites were distributed in 6 sampling areas, being at least 6 km apart from each other. Each sampling area comprised 10 sampling sites, 8 in agricultural fields and, one sampling area situated in a forest and another one situated in a meadow (referred to as semi-natural habitats), to compare the recorded activity levels of the examined agricultural fields to activity levels of habitats known to be used for foraging. To allow direct comparison of bat activity in the different habitats, all sites in an area were sampled simultaneously. In order to consider temporal variability each area was surveyed 5 times, resulting in a total of 300 sampling nights. All sites were located less than 2.5 km away from the closest village and the closest forest of each area, assuring they were within the home range of all native bat species having their roost sites in settlements or forests. The distance of 2.5 km is based on the foraging range of the common pipistrelle (*Pipistrellus pipistrellus*), the species with the shortest maximum distance (2.5 km) between foraging sites and roost sites among the native species (Racey & Swift, 1985; Dietz et al., 2007). Agricultural sampling sites (apple orchards, vineyards, cereal-, and vegetable fields) were chosen to reflect the coverage of the different crops in each area.

At each site, bat activity and nocturnal insect availability was assessed simultaneously, with the insect traps being at least 40 m away from the batcorders to avoid increased and biased bat activity pattern through attraction of the trap light. The recordings of bat activity and the sampling of nocturnal insects were performed from sunset to sunrise. In a few cases light traps did not work the whole night so that individual samples had to be rejected from the analysis. The study was conducted from the beginning of June until the end of August 2008, coinciding with the lactation period for most European bats (Vaughan et al., 1997). All sampling and recording was conducted in nights with temperatures above 16°C at sunset, no rain and a low wind speed (below 10 km/h).

Bat activity measurement

Acoustic measurement of bat activity is as a reliable estimate of foraging activity (Russo & Jones, 2003). Bat activity was recorded by using 10 automatic stationary bat detector systems, so-called batcorders (ecoObs GmbH, Nürnberg, Germany) a method suitable to address spatial and temporal variation in bat activity pattern (Stahlschmidt & Brühl, 2012a). Batcorders were installed at a height of 3.5 m above ground and adjusted to the system's standard settings (Runkel, 2008). The sampling points were chosen in a way that assured uncluttered acoustic space within the detection radius of the system, i.e. 10 m (Runkel, 2008). The activity was measured as the number of recorded call-sequences per night. The software packages bcAnalyse and bcDiscriminator (ecoObs GmbH, Nürnberg, Germany) were used to identify the calls to species level whenever possible. For statistical analyses the individual bat calls were assigned to the following species groups since it was not possible to identify all calls with sufficient probability: the genera *Pipistrellus* and *Myotis* and the group *Eptesicus-Nyctalus*.

Insect sampling

Parallel to the bat recording, we measured the availability of nocturnal aerial insects using unattended light traps. Each light trap consisted of two ultraviolet fluorescent tubes, two crossed acrylic glasses and a plastic bowl filled with two litres of water and three drops of an odourless detergent to reduce surface tension and therefore minimize the escaping of caught insects. Light traps were positioned at least 30 m within the crop field and installed at a height of 1.8m. To assure that only nocturnal insects were sampled, the traps were automatically activated at dusk and deactivated at dawn. Insects other than Diptera or macro-moths were identified to order, Diptera to sub-order and macro-moths to family level. For the results on the nocturnal invertebrate communities we refer to Hahn et al. (submitted). Furthermore, insect size was measured individually and insects were assigned to defined size classes. The prey size suitable for *Pipistrellus*-group is reported to be around 3 mm on average (Barlow, 1997) and mainly less than 5 mm (Beck, 1995). Thus, the main prey size was considered to be 2-5 mm. The species of the *Eptesicus-Nyctalus* group differ in their preferred prey, but all of them include small Diptera (the most frequently recorded insect group in that study) in their diet and generally seem to consume different insects in the proportions encountered (Dietz et al., 2007 and references therein). Therefore insects larger than 2 mm of all orders were considered as potential prey for *Eptesicus-Nyctalus*. Not all recorded *Myotis* species are aerial

hunters and their prey could not be assessed by the applied insect trapping method. Since it was not possible to identify all *Myotis* calls with sufficient probability to species level and, consequently to assign them to groups with similar prey preferences, they were excluded from this analysis.

Statistical Analysis

Permutational multivariate analysis of variance (PERMANOVA Anderson, 2001) was used to assess differences in (1) activities of the bat groups (*Pipistrellus*, *Eptesicus-Nyctalus*, *Myotis*) between the different habitat types (forest, forest edge, open landscape), (2) activities of the bat groups between the examined open landscape habitats (meadow, vineyard, cereal fields, vegetable fields, orchards), and (3) the differences in nocturnal insect availability (insects of the size class 2-5 mm, all insects) between the habitats (forest, forest edge, meadow, vineyard, cereal fields, vegetable fields, orchards). The Euclidean dissimilarity measure was used as the distance metric with 999 permutations for the probability tests. The factors (habitat types, open landscape habitats, insect availability) were treated as fixed, the sampling replication were nested within sites. When a factor was identified as significant (at $\alpha = 0.05$), post-hoc pairwise tests (t-test) were conducted, again using 999 permutations. Analyses were conducted using the software packages PRIMER 6 (version 6.1.13) and PERMANOVA+ (version 1.0.3).

Spearman's coefficient correlation was used to explore relationship between site specific and average bat activities of *Pipistrellus* and *Eptesicus-Nyctalus* and availabilities of nocturnal insects of the size class 2-5 mm and total number of insects, respectively. These analyses were conducted using SPSS ver. 17 (SPSS, Chicago, USA).

RESULTS

Bat activity

In 300 sampling nights a total of 24,012 call sequences were recorded, corresponding to 14 species (Tab. 1). About 66.6% of them were assigned to *Pipistrellus*, 26.3% to *Eptesicus-Nyctalus*, 6.1% to *Myotis*, and 0.3% to *Plecotus*. *Barbastella barbastellus* was only recorded 3 times. The remaining 0.6% sequences were unidentifiable and thus excluded from the analysis. By far the most detected species was *Pipistrellus pipistrellus* with 65.0% of all the recorded call sequences.

Table 1. Total and average number of bat call sequences per habitat. Average numbers of sequences per habitat were calculated as the mean of all sampling night (n = 5 per site) and all sites per habitats (forest: n = 6; forest edge: n = 2; meadow: n = 6; vineyard: n = 13; orchard: n = 5; vegetable: n = 19; cereal: n = 9).

Habitat type Habitat	forest		Forest edge		meadow		vineyard		orchard		vegetable		cereal	
	total	average	total	average	total	average	total	average	total	average	total	average	total	average
<i>Pipistrellus pipistrellus</i>	1108	36.9	5716	571.6	602	20.1	615	9.5	3511	140.4	2789	29.4	1263	28.1
<i>Pipistrellus nathusii</i>	7	0.2	47	4.7	16	0.5	17	0.3	34	1.4	88	0.9	39	0.9
<i>Pipistrellus pygmaeus</i>	1	<0.1	8	0.8	2	0.1	-	-	12	0.5	13	0.1	4	0.1
<i>Pipistrellus</i> spp.	18	0.6	18	1.8	7	0.2	3	<0.1	35	1.4	21	0.2	4	0.1
<i>Pipistrellus</i> (total)	1134	37.8	5789	578.9	627	20.9	635	9.8	3592	143.7	2911	30.6	1310	29.1
<i>Eptesicus serotinus</i>	199	6.6	400	40.0	371	12.4	137	2.1	132	5.3	666	7.0	341	7.6
<i>Eptesicus nilssonii</i>	54	1.8	19	1.9	75	2.5	4	0.1	4	0.2	53	0.6	12	0.3
<i>Nyctalus noctula</i>	121	4.0	214	21.4	91	3.0	31	0.5	43	1.7	400	4.2	227	5.0
<i>Nyctalus leisleri</i>	25	0.8	79	7.9	19	0.6	17	0.3	15	0.6	32	0.3	38	0.8
<i>Eptesicus / Nyctalus</i> spp.	349	11.6	290	29.0	424	14.1	174	2.7	128	5.1	800	8.4	341	7.6
<i>Eptesicus / Nyctalus</i> (total)	748	24.9	1002	100.2	980	32.7	363	5.6	322	12.9	1951	20.5	959	21.3
<i>Myotis mystacinus brandtii</i>	147	4.9	37	3.7	15	0.5	18	0.3	32	1.3	95	1.0	24	0.5
<i>Myotis daubentonii</i>	95	3.2	34	3.4	18	0.6	2	0.0	8	0.3	30	0.3	15	0.3
<i>Myotis bechsteini</i>	112	3.7	30	3.0	6	0.2	7	0.1	-	-	5	0.1	-	-
<i>Myotis nattereri</i>	93	3.1	23	2.3	10	0.3	26	0.4	29	1.2	28	0.3	4	0.1
<i>Myotis myotis</i>	8	0.3	4	0.4	2	0.1	5	0.1	-	-	55	0.6	5	0.1
<i>Myotis</i> spp.	176	5.9	59	5.9	20	0.7	19	0.3	29	1.2	119	1.3	28	0.6
<i>Myotis</i> (total)	631	21.0	187	18.7	71	2.4	77	1.2	98	4.0	332	3.5	76	1.7
<i>Plecotus austriacus</i>	2	0.1	2	0.2	1	<0.1	47	0.7	-	-	8	0.1	3	0.1
<i>Barbastella barbastellum</i>	1	<0.1	2	0.2	-	-	-	-	-	-	-	-	-	-

Apart from the common pipistrelle, Nathusius's bat (*Pipistrellus nathusii*) and the midge bat (*Pipistrellus pygmaeus*) of the genus *Pipistrellus* were detected (Tab. 1). In average, the highest numbers of total *Pipistrellus* call sequences were recorded at the forest edges, the lowest numbers above the vineyards (Tab. 1). Relatively high numbers were detected in the orchards while forests, meadows, cereal and vegetable fields were used to similarly extents (Tab. 1). The following species of the group *Eptesicus-Nyctalus* were recorded: the serotine (*Eptesicus serotinus*), the northern bat (*Eptesicus nilssonii*), the noctule (*Nyctalus noctula*), and Leisler's bat (*Nyctalus leisleri*). On average, the highest numbers of call sequences of all *Eptesicus-Nyctalus* were recorded at the forest edges. For all species of that group similar activities were detected in the forests and open landscape habitats (Tab. 1). The genus *Myotis*

was represented by the whiskered bat (*Myotis mystacinus / brandtii*), Daubenton's bat (*Myotis daubentonii*), Bechstein's bat (*Myotis bechsteinii*), Natterer's bat (*Myotis nattereri*) and the greater mouse-eared bat (*Myotis myotis*). All *Myotis* species showed a strong preference for forest and forest edges habitats with the exception of the greater mouse-eared bat with slightly higher activity over vegetable fields (Tab. 1). Bechstein's bat was almost exclusively recorded in forests and at forest edges (Tab. 1). Average number of call sequences of the grey long-eared bat (*Plecotus austriacus*) was highest over vineyards (Tab. 1). The barbastelle (*Barbastella barbastellum*) was only recorded twice at forest edges and once in a forest (Tab. 1).

Table 2. Results of pairwise comparisons (PERMANOVA) of the number of calls of the 3 bat groups (*Pipistrellus*, *Eptesicus-Nyctalus*, *Myotis*) between the different open landscape habitats (meadow, vineyard, orchard, vegetable, cereal). Significant values are in bold.

	<i>Pipistrellus</i>		<i>Eptesicus-Nyctalus</i>		<i>Myotis</i>	
	P(perm)	t	P(perm)	t	P(perm)	t
Vineyard - meadow	0.001	5.341	0.001	3.593	0.179	1.490
Vineyard - vegetable	0.001	3.076	0.002	3.417	0.027	2.347
Vineyard - cereal	0.001	3.084	0.002	3.343	0.659	0.552
Vineyard - orchard	0.002	2.221	0.051	2.228	0.059	1.871
Orchard - cereal	0.077	1.501	0.259	1.136	0.425	0.927
Orchard - meadow	0.032	1.296	0.132	1.604	0.613	0.550
Orchard - vegetable	0.055	2.181	0.272	1.102	0.976	0.054
Cereal - meadow	0.481	0.721	0.324	1.050	0.689	0.477
Cereal - vegetable	0.898	0.157	0.908	0.127	0.199	1.372
Meadow - vegetable	0.451	0.836	0.158	1.430	0.464	0.762

The differences in activity levels between habitat types (forest, forest edge, open landscape) were significant for the groups *Pipistrellus* and *Eptesicus-Nyctalus* (PERMANOVA: $P > 0.005$ in both cases), a pair-wise comparisons (PERMANOVA) showed no differences between open landscape and forest ($P = 0.883$ and $P = 0.401$, respectively), between forest edge and forest ($P = 0.036$ and $P = 0.062$, respectively) but between forest edge and open landscape ($P = 0.005$ and $P = 0.003$, respectively) caused by the high number of recorded call sequences for both groups at the forest edge habitats (Tab. 1).

Significant differences in activity patterns between the different habitats of the open landscape were also found for the groups *Pipistrellus* and *Eptesicus-Nyctalus* (PERMANOVA: $P = 0.011$ and $P = 0.005$, respectively). Pair-wise comparisons revealed that

the vineyards differ in number of *Pipistrellus* call sequences from all other open landscape habitats (Tab. 2). The same pattern was found for *Eptesicus-Nyctalus* with the one exception that there was no difference in activity between the vineyards and orchards (Tab. 2).

Activity levels between habitat types were different for *Myotis* (PERMANOVA: $P = 0.001$). Pair-wise comparison (PERMANOVA) demonstrated differences between open landscape and forest ($P = 0.001$), between open landscape and forest edge ($P = 0.003$) but not between forest edge and forest ($P = 0.918$) which could be attributed to the low activity levels recorded at the open landscape. No differences were found between *Myotis* call sequences at the different open landscape habitats (PERMANOVA: $P = 0.162$), which were on average 5-18 times lower compared to those in the forests and at the forest edges (Tab. 1).

When comparing the summed bat activity pattern for the five nights of all examined habitats which were simultaneously recorded in each sampling area, the highest activity levels were recorded at forest edges (sampling areas 1 and 2), over vegetable fields (sampling areas 3 and 4), an orchard (sampling area 5), and within a forest (sampling area 6).

Food availability

In total 109,264 insects with body size larger than 2 mm were trapped in 281 sampling nights (70,735 of them were assigned to the size class 2-5 mm). More than 70 % of the sampled insects were assigned to the order Diptera. In average, the highest numbers of insects larger than 2 mm were found at the forest habitats (Tab. 3). Numbers of insects of the size class 2-5 mm were highest at the vegetable fields and forest (Tab. 3). For both size the lowest numbers of insects were found at the vineyards (Tab. 3). Availability of total nocturnal insects larger than 2 mm and insects of the size classes 2-5 mm, representing suitable prey for *Eptesicus-Nyctalus* and *Pipistrellus*, respectively, differed significantly between the habitats (PERMANOVA: $P = 0.002$ and $P = 0.001$, respectively). Pair-wise comparisons revealed that this could be attributed to the vineyards which differed by lower insect abundances from the forest, meadow and the other crops while no differences between the other three habitats were found (Tab. 4).

Table 3. Average numbers of nocturnal insects per habitat. Average numbers of insects per habitat were calculated as the mean of all sampling nights (n = 4-5 per site) and all sites per habitats (forest: n = 6; meadow: n = 6; vineyard: n = 14; orchard: n = 5; vegetable: n = 19; cereal: n = 10).

Size class	Forest	Meadow	Vineyard	Orchard	Vegetable	Cereal
> 2 mm	644	390	161	386	496	372
2-5 mm	353	248	82	271	354	262

Table 4. Results of pairwise comparisons (PERMANOVA) of numbers of nocturnal insects (insects larger than 2 mm; insects sized between 2 and 5 mm) between the different habitats (forest, meadow, vineyard, orchard, vegetable, cereal). Significant values are in bold.

	Insects larger than 2 mm		Insects 2-5 mm	
	P(perm)	t	P(perm)	t
Vineyard - meadow	0.001	3.715	0.001	4.063
Vineyard - vegetable	0.001	4.370	0.001	4.435
Vineyard - cereal	0.001	4.129	0.001	4.154
Vineyard - orchard	0.001	6.119	0.002	6.638
Vineyard - forest	0.001	9.249	0.001	9.331
Orchard - cereal	0.655	0.474	0.574	0.605
Orchard - meadow	0.756	0.431	0.742	0.418
Orchard - vegetable	0.163	1.387	0.183	1.357
Orchard - forest	0.055	3.139	0.052	2.726
Cereal - meadow	0.984	0.022	0.847	0.179
Cereal - vegetable	0.190	1.412	0.274	1.178
Cereal - forest	0.051	2.337	0.178	1.437
Vegetable - forest	0.620	0.545	0.816	0.230
Meadow - vegetable	0.306	1.084	0.314	1.087
Meadow - forest	0.074	1.928	0.142	1.575

A significant positive correlation between site specific *Pipistrellus* activity and insect availability of the size class 2-5 mm ($r_s = 0.340$, $p = 0.007$, $n = 60$; Fig. 1a) and, respectively, site specific *Eptesicus-Nyctalus* activity and all insects larger than 2 mm was found ($r_s = 0.484$, $p = 0.001$, $n = 60$, Fig. 1b).

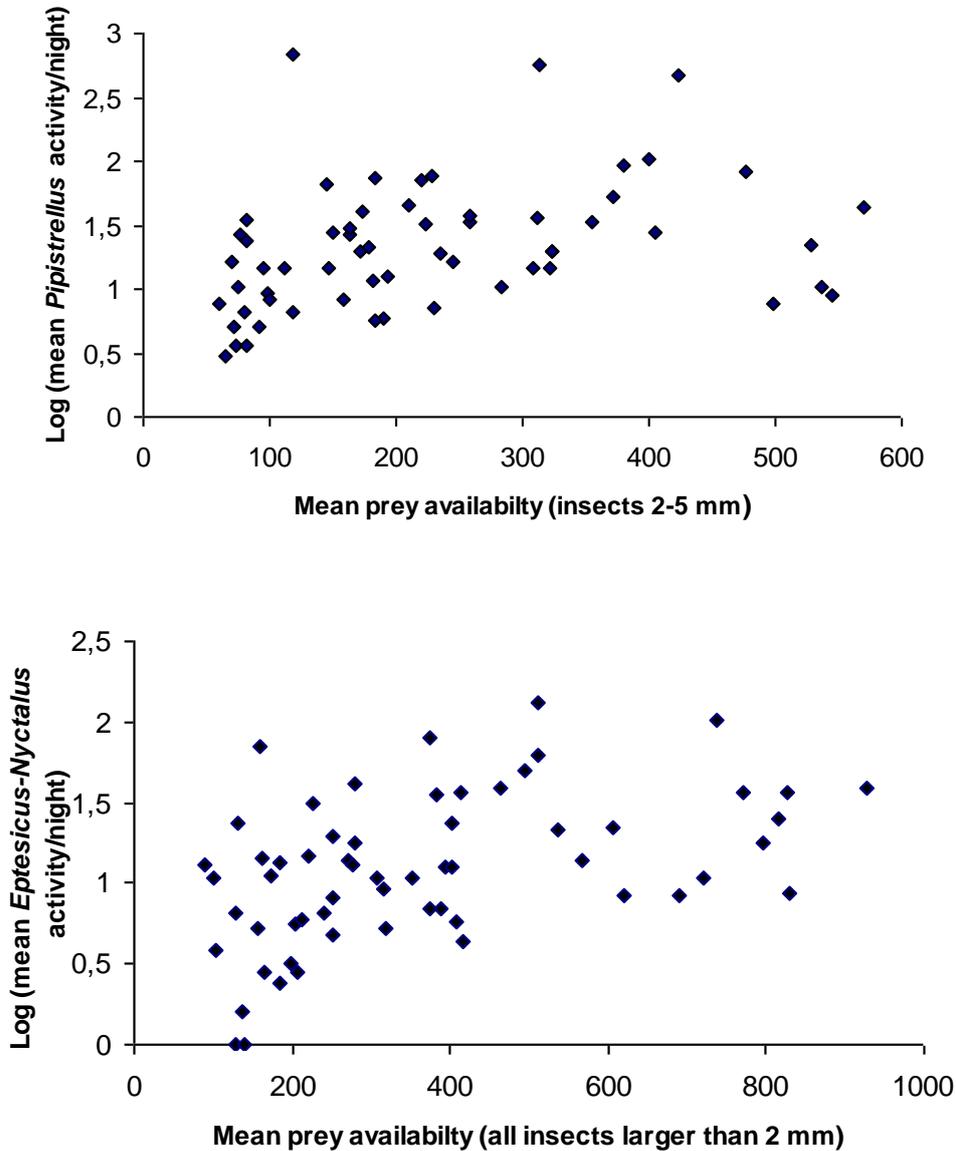


Figure 1. Scatter plots of site-specific average ($n = 5$ nights per site) bat activity of *Pipistrellus* (a) and *Eptesicus-Nyctalus* (b) against site-specific availability of the corresponding prey groups.

DISCUSSION

Farmland is the vastest terrestrial wildlife habitat in Europe, covering 43% of the EU member states' surface area (Geiger et al., 2010). For bats, however, little is known about the role of agricultural crop fields as foraging habitats. In contrary, the use of freshwater habitats or deciduous forests, both generally represent only small portions of most European landscapes, are well studied. Some studies have reported an avoidance of intensively managed agricultural fields by bats (Walsh & Harris, 1996; Vaughan et al, 1997). However, results of Vaughan et al (1997) showed that bat activity levels over arable land in Great Britain were statistically lower for most bat species compared to their activities over water surfaces (i.e. rivers and lakes) but were comparable to the examined non-arable terrestrial habitats (different kinds of grassland and woodland). Given that water habitats are rare within most European landscapes while in contrast arable land constituting more than 40 % of the available habitat (Walsh & Harris, 1996), the predominant arable land, even if disproportionately more scarcely used by bats, may play an important and currently underestimated role as a foraging habitat. Wickramasinghe et al. (2003) compared bat activity across conventional and organic agricultural land and recorded higher activity on organic farms. However, these differences were also only found over water but not over land habitats (Davy et al., 2007). Even higher bat activity levels were demonstrated on conventional farms when compared to farms using less intensive agricultural practices (Fuentes-Montemayor et al., 2011). Relatively large numbers of foraging attempts were recorded in some arable fields (Russo & Jones, 2003). However, none of the aforementioned studies provides details about the crops in order to allow any conclusion about potential exposure of bats to pesticides. The present study is the first detailed investigation of the diversity and activity of European bats in different agricultural crops.

Bat activity

All 14 bat species recorded in the different habitats of the six sampling areas were also detected over agricultural fields, among them the northern bat, a species reported locally as facing extinction and the rare barbastelle which was not yet recorded in this region of Rhineland Palatinate (König & Wissing, 2007). Activity at a sampling site does not necessarily reflect its quality as a foraging habitat since quality is also reflected by the number of bat individuals present which also depends on roost site availability and the distance to

them. Therefore comparisons of site-specific activity levels of different habitat types on a large spatial scale are problematic (Hayes, 2000). However our study design with several sampling sites in different habitats grouped in a sampling area within the home-range to potential roost sites (both housing and forests) for all occurring species, allows the direct comparison of activity levels between the different habitats.

The activity levels of the recorded species of the genera *Pipistrellus*, *Eptesicus* and *Nyctalus*, all of them being predominately aerial hawker, did not significantly differ between agricultural sites, forests and meadow habitats. Higher activity levels over agricultural fields than those in the simultaneously examined meadows and forests could even be demonstrated in several cases (fruit orchards, vegetable fields).

The activity levels of both groups (*Pipistrellus* and *Eptesicus-Nyctalus*) comprising species that are predominantly aerial hawkers were correlated with suitable prey insect availability indicating that they use the agricultural sites for foraging. In accordance to the significant lower insects abundances found at the vineyards activity levels of the aerial hawkers were also significantly lower over there compared to all other crop types.

In contrast, higher activity levels in the forests and significantly reduced activity in the open landscape were found for the *Myotis* species. Most of the recorded *Myotis* species are known to take their prey mainly (Natterer's and Bechstein's bat) or at least partly (Whiskered and Brandt's bat) by gleaning from vegetation (Dietz et al., 2007 and references therein). Bats using this foraging strategy are more adapted to high-clutter environments such as forests (e.g. in regards to their echolocation), but not to open landscape habitats (Aldridge & Rautenbach, 1987). Exceptions are the greater mouse-eared bat which almost exclusively feeds on carabid beetles and Daubenton's bat, a species adapted to take prey from water surfaces (Dietz et al., 2007 and references therein).

All examined bat groups showed remarkably high activity levels over agricultural fields located next to forests. Forest edges in general are known to be used for foraging by bat species that avoid navigating through structurally complex habitats as well as those that avoid the open landscape (Walsh & Harris, 1996; Morris et al. 2010).

Food availability

Abundances of insects of the examined size classes did not differ between the forest, meadow and most agricultural habitats. This appears to be in contrast to other studies reporting insect abundances and diversity being negatively associated with agricultural intensification (e.g. Benton et al., 2002; Wickramasinghe et al., 2004). We only compared abundance of nocturnal

insects with more than 70% being Diptera. In a study by Nielsen et al. (1994) the occurrence of Diptera was not significantly impacted by pesticide use and, while tillage has been reported as a disturbance factor for terrestrial Diptera, some species are even specialized on the initial stages of succession after tillage (Frouz, 1999). Thus some Diptera species may be less affected by agricultural intensification and occur in high abundances in the crop fields. The main factors affecting the occurrence of Diptera with terrestrial larval stages are the organic matter content and the moisture of the soil (Frouz, 1999). The soils of vegetable fields are especially rich in organic matter due to the remnants of the former crops (up to 3 different vegetable cultures per year). In combination with the presence of permanently wet soils due to irrigation, vegetable fields appear to provide the most suitable conditions of the examined crops for Diptera leading in several cases to insect abundances even exceeding those measured simultaneously at the nearby forest sites. The soils of the cereals fields are also relatively rich in organic matter due to the remnants of the former crops while the orchards are poorer in this regards the soils there are more humid due to the shade of the apple trees. Vineyards, however, do not providing conditions very attractive for most Diptera since their soils are rather dry due to their exposed position.

Potential exposure to pesticides

Given the high bat activity levels recorded at several agricultural sites and the availability of suitable prey insects, an uptake of pesticides through consumption of potentially contaminated food items after pesticide application is likely. Especially high bat activity levels were recorded in several apple orchards, a crop known for high pesticide input (Roßberg, 2007). Because of the vegetation structure suitable for gleaning, orchards were the only crop where Natterer's and Brandt's bat were recorded on a regular basis. Since the estimation of the exposure requires information on pesticide residues on bat-specific food items, a follow-up study (Stahlschmidt & Brühl, 2012b) was performed in one of the apple orchards where high bat activity levels were demonstrated. According to the preferences of the recorded bat guilds the residue pattern of different nocturnal arthropod groups were examined following applications of insecticides. The highest residue values were measured on foliage-dwelling arthropods which may result in a risk for all bat species that, even to a small extent, include foliage-dwelling arthropods in their diet (Stahlschmidt & Brühl, 2012b).

Considering the high bat activity levels recorded over several vegetable fields indicating a good foraging habitat and the massive pesticide input in these crops (Roßberg, 2007), a study of pesticide residue patterns on nocturnal arthropods is strongly suggested to get a realistic

estimate for the risk of pesticide exposure. The average number of call sequences per night of the greater mouse-eared bat, a species almost exclusively feeding on carabid beetles (Beck, 1995), was highest above the vegetable fields. Ground-dwelling arthropods such as carabid beetles may exhibit high pesticide residues especially after ground-directed applications in the afternoon. A massive die-off of juvenile greater mouse-eared bats which was attributed to the application of an organophosphate to potato fields and apple orchards in Germany (Hoffmann, 1991) already demonstrated that this species is threatened by pesticide exposure. While in the orchards most of the airborne small insects were non-Diptera such as small moths (Hahn et al., submitted), Diptera were the predominant group in the vegetable fields. Since it has been shown that Diptera larvae can accumulate significant amounts of chemicals (Eitminavichiute et al., 1982; Park et al., 2009), residue patterns in vegetable fields may differ from those measured in the orchard. Research is required to examine if such an accumulation of modern and less persistent pesticides takes place in Diptera developing in agricultural soils, especially in vegetable fields where wet soils may increase the contact of the larvae with pesticides.

Bat activity was rather low over the vineyards with the exception of the grey long-eared bat. While availability of nocturnal insects in general was lower in vineyards compared to the other agricultural habitats, higher abundances of nocturnal moths of the family Noctuidae (Hahn et al., submitted), on which the grey long-eared bat is almost exclusively preying (Bauerová, 1982), were recorded. In the residue study performed in the apple orchard (Stahlschmidt & Brühl, 2012b) large moths exhibited the lowest pesticide residues of all examined arthropods groups, revealing the lowest risk for bat species mainly feeding on them. Therefore, similar low residue pattern on the moths and a low risk for the grey long-eared bat feeding on them are expected in the vineyards.

Remarkably high activity levels of all examined bat groups were detected over agricultural fields located next to forests. Given that in agricultural landscapes most forest edges are situated next to crop fields, a thorough examination of the potential pesticide exposure is necessary and special risk mitigation methods for those habitats may be required. Forest edges function as windbreaks which potentially could concentrate large densities of contaminated insects after pesticide application. The northern bat and the barbastelle were in this study predominantly recorded at the forest edges. Both are rare species and a potential risk due to pesticide exposure could even have severe impacts on their populations. Research is also required if Bechstein's bat and the brown long-eared bat (*Plecotus auritus*), both forest inhabiting bats exclusively taking their prey by gleaning, are using orchards situated next to

forests for foraging since a high risk is expected due to the high residue values of foliage-dwelling arthropods in orchards (Stahlschmidt & Brühl, 2012b).

Conclusion

The present study demonstrated that abundances of suitable prey insects for aerial hunting bats in orchards, vegetable and cereal fields are comparable to nearby forests and meadows, the latter known to be used as foraging habitats by bats. Since high bat activity was recorded in the orchards and arable fields, crops that are known for elevated pesticide inputs, an exposure through ingestion of pesticide contaminated insects is especially likely there. The following scenarios indicate a risk of pesticide exposure for bats: gleaners foraging in orchards, bats preying on soil arthropods in vegetable fields, aerial hawkers feeding on Diptera over vegetable fields, and bat species foraging along forest edges situated next to agricultural fields. In addition to studies on the pesticide contamination of bat food items as a basis for the development of a realistic risk assessment approach for this group, telemetry studies are needed to gain insights in individual foraging patterns in agricultural habitats.

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Appendix IV Nocturnal insects in different crops and the potential off provision for two bat species

**Nocturnal insects in different crops and the potential food
provision for two bat species**

Melanie Hahn, Carsten A. Brühl & Peter Stahlschmidt

Manuscript

Nocturnal insects in different crops and the potential food provision for two bat species

Melanie Hahn*, Carsten A. Brühl & Peter Stahlschmidt

Institute for Environmental Sciences, University of Koblenz-Landau, Fortstraße 7, D-76829

Landau, Germany

*Correspondence author. E-mail: hahn@uni-landau.de

ABSTRACT

In agro-ecosystems insects not only form a great part of the biodiversity and play a central role in the maintenance of ecosystem services but also serve as food for other organisms. However, the assessment of abundance and community composition of insects in different crops has only just begun. In this study we focused on the abundance and community composition of nocturnal insects in three crops (cereal, vegetable and vine) in Southern Germany using light trapping. Furthermore, the vegetation of the agricultural sites and the adjoining field margins was assessed to gain insight into the availability of (lepidopteran) host plants. In total, 24,609 insects belonging to 12 orders were sampled and identified. An analysis of the abundances per order revealed significant differences between vineyards and the arable crops which were predominantly caused by the orders Lepidoptera and Diptera. In vineyards the number of Lepidoptera per sample was two- to threefold higher than in the arable crops, probably caused by the greater availability of host plants and no insecticide applications while the Diptera were about 3.5 times more abundant in the arable crops. Lepidoptera and Diptera are essential prey organisms for a range of bat species and differences in their occurrence might influence the prey availability for bats which is exemplarily discussed for the pipistrelle (*Pipistrellus pipistrellus*) and the grey long-eared bat (*Plecotus austriacus*). We conclude that there is a need to take greater account of the prey availability for bats and that the creation of plant species rich strips of (permanent) vegetation within or adjoining to the agricultural fields in combination with a reduced pesticide input could at least improve the abundance of Lepidoptera.

INTRODUCTION

Croplands and pastures constitute one of the greatest terrestrial biomes on earth (Foley et al. 2005) and in Europe agriculture is the leading land-use (Stoate et al. 2009). In agricultural landscapes, insects are of special importance: Firstly, insects form a great part of the biodiversity of agro-ecosystems and therefore of special importance for the conservation of biodiversity. Secondly, insects benefiting the ecosystem and the agricultural production by maintaining ecosystem services like pollination (Allen-Wardell et al. 1998) and biological pest control (Power 2010) Thirdly, they are substantial food sources for species of higher trophic levels such as insectivorous birds and bats.

However, there is increasing evidence that several insect groups are declining in agricultural landscapes (e.g. Wilson et al. 1999; Fox 2012). These declines are often associated with the effects of agricultural intensification (Benton et al. 2002). Intensified agriculture is characterized by an enhanced use of pesticides and mineral fertilizers as well as an enlargement of field size associated with the loss of semi-natural habitats (Stoate et al. 2001). Detrimental effects of the application of agrochemicals (especially pesticides) and the loss of habitats have been documented for several taxa, including Lepidoptera, Diptera and Coleoptera (Wilson et al. 1999). Concerning agrochemicals, adverse effects on insects include direct toxicity caused by insecticides as well as the loss of host plants due to herbicide or fertilizer applications are possible (exemplified by Longley & Sotherton 1997 for butterflies). A study of common, larger moths in Britain revealed that two thirds of the considered species declined in their population size in the past 35 years. Agricultural intensification is considered as one of the main causes for these declines (Conrad et al. 2006).

Insect chick food depletion affects the breeding success of several bird foraging in the agricultural landscape such as the Grey Partridge (*Perdix perdix*) and the corn bunting (*Miliaria calandra*) (Rands 1985; Brickle et al. 2000). Since all Central European bats, which have suffered massive declines in the last 50 years, are insectivorous a decline in insects is likely to affect them (Wickramasinghe et al. 2004).

Agricultural management practices such as pesticide applications are related to the type of cultivated crop (Stoate et al. 2009). So far the abundance of insects within different agricultural crops is hardly understood (Holland et al. 2012). A first insight in this theme is given by Holland and co-workers (2012) who found differences in community patterns and abundances of insects comparing different crops which also resulted in differences in the Grey Partridge chick-food index. Such results can be used to improve the management of

crops that indicate a short-coming in prey-availability via agri-environment schemes (Holland et al. 2012).

In this study we focused on the nocturnal insect communities. Nocturnal insects are the main prey for all European bat species (see e.g. Vaughan 1997). We sampled insects in three crops (cereal fields, vegetable fields, and vineyards) using light traps and compared their abundance and community composition. Furthermore, we studied one group, the Noctuidae (Lepidoptera) in detail and analyzed the potential value of the cropped area and the adjoining field margin as breeding habitat by examining the availability of host plants. These results were discussed regarding the food demands of two bat species.

MATERIALS AND METHODS

Study design

The study was conducted in an agricultural landscape around Landau in Southern Rhineland-Palatinate, Germany, where a wide variety of crops is cultivated. We sampled nocturnal insects in five vineyards (vin), five vegetable fields (veg), and five cereal fields (cer) from June to August 2008. The chosen crops were common in the considered landscape and differ in the pesticides regime and the occurrence of non-crop plants: Vine is a perennial crop and the sampled vineyards had rows of permanent vegetation (grasses and herbs) established in every second vine row to reduce soil erosion. The crop specific Treatment Index, calculated by the NEPTUN-project to quantify pesticide usage of German farmers, indicates low herbicide inputs in vineyards (Roßberg 2009b). Furthermore, the sampled vineyards were not treated with insecticides since two important pest species (Lepidoptera: *Lobesia botrana* and *Eupoecilia ambiguella*) were targeted with specific pheromones (Roßberg 2009b). In cereal and vegetable fields non-crop plants are removed by soil cultivation and herbicide usage in the whole field. Overall, Treatment Indices for both herbicides and insecticides were found to be higher in vegetable than in cereal fields (Roßberg et al. 2002; Roßberg 2009a).

The sample sites were distributed in five regions (distances between the regions: ca. 4.5 to 13km) with two to five sample sites per region. In each region the sample sites were at least 35 to 300m apart from each other. Sites were not situated next to hedges or forest edges to exclude effects of shelter or additional food sources provided by these structures on the insect communities. All sample sites of a region were sampled simultaneous but in a few cases single light traps did not work the whole night so some individual samples have to be

rejected. Overall, 80 complete samples were available, 26 samples in cereal fields, 29 in vegetable fields, and 25 in vineyards (per site: four to six samples).

At each sample site one light trap was positioned 20-30m in the crop and ran the complete night (min. temperature: 10°C, hourly mean wind speed: < 5m/s). The light traps, consisting of two black light lamps (each 3.4W), two crossed acryl glasses (each 25x40cm) and an attached plastic bottle (diameter: 27cm, depth: 10cm) filled with two litres of water and an odourless detergent (Polyoxyethylen-sorbitan-monooleat) were activated at dusk and deactivated at dawn by an automatic trigger (modified bioform light trap, bioform, Nürnberg, Germany). In previously tested trapping systems without water, some insects (especially Diptera) could be sampled only in low numbers although they were attracted in high numbers by the light (pers. observation). Hence, trapping system was modified and water was added to assure a better representation of this group. The light traps were installed at a height of 1.8m.

Taxonomic identification

Insects were identified to order, macro-moths and Pyralidae to family and Noctuidae (Lepidoptera) to species. Identification to species level was possible in most cases (89%) but individual Noctuidae were not identified if they had lost most of their scales in the collection fluid. These individuals were excluded from the statistical analysis which required detailed taxonomical information.

Vegetation and host plants

Vegetation surveys were conducted in the agricultural sites and their margins (= strips of permanent vegetation adjoining to the agricultural sites) by a qualitative assessment of occurring plant species. Overall, 80% of the assessed plant taxa were classified to species; the remaining 20% had been damaged via mowing or agricultural cultivation which only allowed identification to genera level. Based on the mowing of most margins, grasses were not classified to species level.

For the analysis of host plant availability, the vegetation data were pooled for each crop and compared with known caterpillar host plants for the Noctuidae trapped in each crop (Ebert 1997a; b; 1998).

Statistical analysis

The abundance and vegetation data were analyzed using the program Primer (version 6.1.13) and the PERMANOVA+ add-on (version 1.0.3). Since the insect data were not normally

distributed permutational ANOVAs (PerANOVA) and permutational MANOVAs (PerMANOVA) were used (Anderson 2001; Anderson et al. 2008). For the insect data PerANOVAs and PerMANOVAs include two factors (crop type, sample site) in a nested design: While the crop type was treated as fixed factor, the sample sites were nested in the crop type. For the vegetation data only the crop type was included since there was one vegetation assessment per sample site. If the results of the permutational (M)ANOVA showed significant differences in the factor crop type pairwise permutational (M)ANOVAs for this factor were calculated. In the main tests as well as the pairwise tests significant results were evaluated on the basis of 999 permutations as proposed for an α -level of 0.05 (Anderson 2001; Anderson et al. 2008). The underlying resemblance matrices were calculated with the Euclidian distance (PerANOVA) or the Bray-Curtis distance (PerMANOVA).

The analysis of similarity (SIMPER) is a possibility to determine how much individual taxa contribute to the separation between two groups of samples (Clarke & Gorley 2006). For our data we used for the calculation the Bray-Curtis similarity.

RESULTS

In total, 24,609 insects belonging to 12 orders were sampled and identified. There were no differences between the crops concerning total insect number per sample (PerANOVA, $P(\text{perm})= 0.272$, Table 1). Three orders, Lepidoptera, Diptera and Coleoptera formed roughly 80% of the trapped insect numbers (Figure 1).

Regarding the insect numbers per order the community composition differed significantly between the crops (PerMANOVA, $P(\text{perm})=0.001$). Pairwise comparisons revealed that this could be attributed to the vineyards which differed from the arable crops (PerMANOVA: $P(\text{perm})_{\text{vin-cer}}= 0.004$; $P(\text{perm})_{\text{vin-veg}}= 0.006$; $P(\text{perm})_{\text{veg-cer}}= 0.139$). The differences between vineyards and arable crops were predominantly caused by the orders Diptera and Lepidoptera (highest proportions of dissimilarities between the crops (SIMPER): cer-vin: Diptera 53%, Lepidoptera 18%; veg-vin: Diptera: 47%, Lepidoptera: 20%).

Noctuidae was the most abundant macro-moths family in our samples. Hence, this family was studied in more detail. Overall, 362 Noctuidae belonging to 32 species were trapped. In vineyard samples significantly more Noctuidae were trapped whereupon their numbers in vegetable and cereal field samples did not differ significantly (PerANOVA, pairwise comparisons: $P(\text{perm})_{\text{vin-cer}}= 0.005$; $P(\text{perm})_{\text{vin-veg}}= 0.004$; $P(\text{perm})_{\text{veg-cer}}= 0.308$, Table 1). In

total, in vineyards a greater number of Noctuidae species could be trapped than in both arable crops (cer: 13, veg: 16, vin: 24 Noctuidae species). The number of Noctuidae species per sample was also significantly higher in vineyards than in the arable crops (PerANOVA, pairwise comparisons: $P(\text{perm})_{\text{vin-cer}} = 0.016$; $P(\text{perm})_{\text{vin-veg}} = 0.012$; $P(\text{perm})_{\text{veg-cer}} = 0.612$, Table 1). Concerning the species composition of the Noctuidae, there were significant differences between all three crops (PerMANOVA, pairwise comparisons: $P(\text{perm})_{\text{vin-cer}} = 0.008$; $P(\text{perm})_{\text{vin-veg}} = 0.004$; $P(\text{perm})_{\text{veg-cer}} = 0.005$).

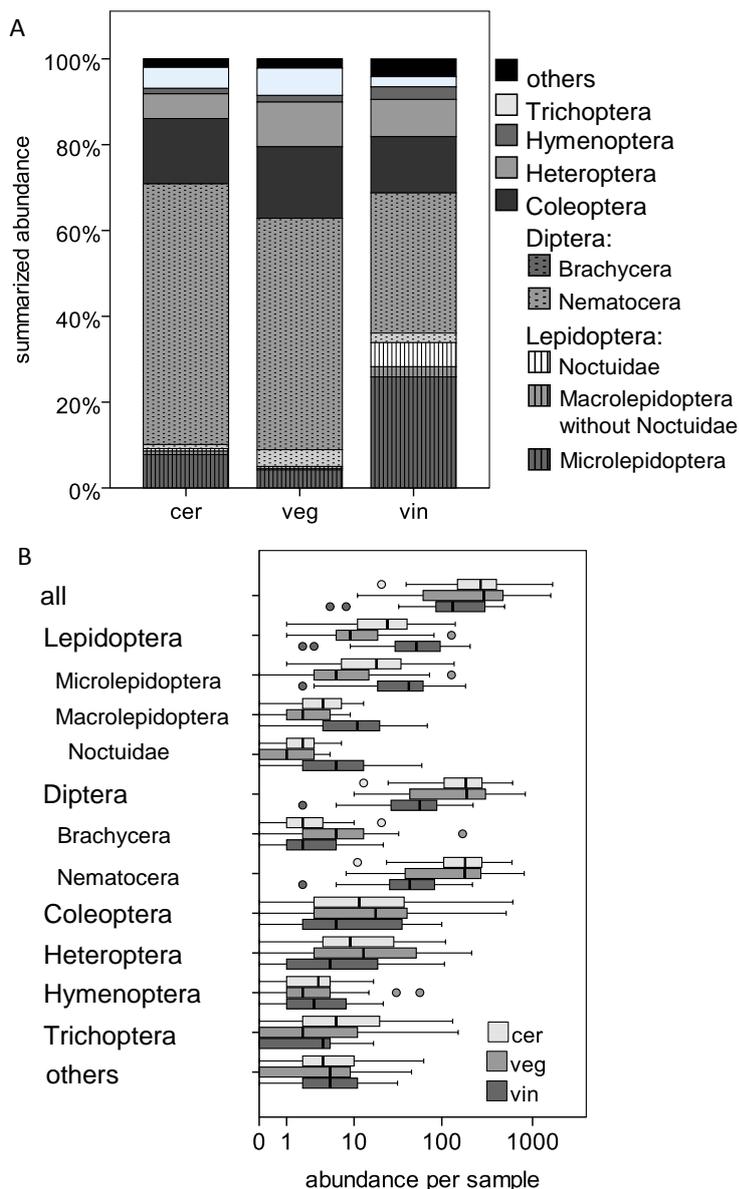


Figure 1: Relative abundance of the trapped insects (A) for the different crops and box plots of the abundances per sample (B, dots represent outliers). In (A) the orders Diptera and Lepidoptera were subdivided into Brachycera, Nematocera and Microlepidoptera, Macrolepidoptera, Noctuidae, respectively. These subgroups are also included in (B). Others: Aphidina, Auchenorrhyncha, Ephemeroptera, Neuroptera, Psocoptera, Psyllina.

Table 1: Comparison of the three crops concerning insect and plant characteristics.

	cer		veg		vin	
	mean	SE	mean	SE	mean	SE
number of insects per sample	351.23	68.16	373.72	72.06	185.60	28.81
number of Diptera per sample	216.69	31.85	216.21	35.30	64.76	9.97
number of Lepidoptera per sample	32.19	5.98	18.69	5.13	62.84	9.81
number of Noctuidae per sample	2.04	0.33	1.69	0.32	10.4	2.78
Noctuidae species per sample	1.31	0.19	1.21	0.22	2.56	0.43
non-crop plant species per site (cropped area)	2	0.63	5.2	1.07	11.2	1.2
non-crop plant species per site (field margins)	6.4	0.93	4.6	0.4	5.8	1.16

Due to the permanent vegetation strips in vineyards a significantly higher number of non-crop plant taxa per site was found in the cropped area compared to the arable crops (PerANOVA, pairwise comparisons $P(\text{perm})_{\text{vin-cer}} = 0.009$; $P(\text{perm})_{\text{vin-veg}} = 0.009$; $P(\text{perm})_{\text{veg-cer}} = 0.075$, Table 1). However, all sites showed a similar number of plant taxa in their margins (PerANOVA, $P(\text{perm}) = 0.362$, Table 1).

A comparison of the host plants of the Noctuidae species trapped in the course of the study with the vegetation (pooled per crop) of the sample sites and the field margins demonstrated that for most species at least one host plant was available (Figure 2). The relevance of the field margins as reservoirs of host plants differed between the crops: In cereal cropping systems, host plants for 60% of the Noctuidae species grew exclusively in field margins. In vineyards the cropped area supplied host plants for nearly 90% of the Noctuidae species trapped there.

DISCUSSION

With crop specific analyses of insects communities it is possible to get a better insight in some of the causes responsible for the recently observed decline of insects and associated species in agricultural landscapes. Our abundance data on the nocturnal insect communities indicated strong differences between the arable crops and the vineyards which could mostly be attributed to the orders Diptera and Lepidoptera.

Many Diptera species are soil-dwelling during their larvae or pupae stage. The abundance of these larvae is higher in wet soils (Frouz 1999). Since vegetable fields are irrigated in our study region, this could benefit the occurrence of Diptera in these fields in comparison to the non-irrigated cereal fields and vineyards. In vineyards, the rows without permanent vegetation are ploughed regularly. It is known that tillage decreases the abundance of dipteran larvae (Frouz 1999). Furthermore, some Diptera avoid a dense and dry litter layer as it is produced by some grasses (Frouz 1999). Such a litter layer is possibly found in the rows with permanent vegetation of the vineyards. Dry soil conditions in combination with periodical tillage (rows without permanent vegetation) and dense grass vegetation (rows with permanent vegetation) might explain the reduced abundance of Diptera in vineyards.

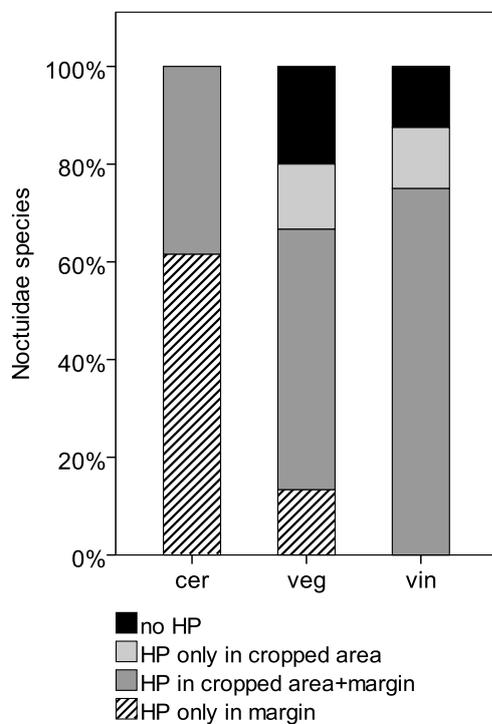


Figure 2: Occurrence of the potential host plants (HP) for the identified Noctuidae species (cer: 13, veg: 16, vin: 24 Noctuidae species). Vegetation data were pooled per crop (N=5)

Both, the Lepidoptera in general and the family Noctuidae in particular showed higher abundances and species richness in vineyards in comparison to both arable crops. A possible explanation for this may be the greater availability of host plants: Firstly, in contrast to the arable crops, at least 50% of the vineyards were covered with vegetation. Since the abundance of some butterfly species was shown to correlate with the abundance of host plants (Saarinen 2002), the area covered with vegetation might have influenced the moth community significantly. Secondly, the number of plant species found in vegetable or cereal fields

(without field margins) was significantly lower in comparison to the vineyards reducing the number of potential host plants. This could be seen as a consequence of the ploughing of the whole cropped area which, thirdly, results in restricted seasonal host plant availability in comparison to vineyards containing strips of permanent vegetation.

Another factor possibly influencing the number of trapped Lepidoptera and Noctuidae are the different pesticide regimes in the crops. For all three crop types certain moth species are considered as pests. While in the vineyards in our study sites the two most important pest species are targeted with pheromones and therefore no insecticides were applied, insecticides are commonly used in the arable fields (Roßberg et al. 2002; Roßberg 2009a) which might affect non-pest species including some moths as well. Laboratory studies have documented lethal and sublethal effects (e.g. weight loss, feeding inhibition) on butterfly caterpillars after exposure to insecticides (Tan 1981; Cilgi & Jepson 1995). The treatment with herbicides could also have a negative impact since it reduces the availability of lepidopteran host plants (Longley & Sotherton 1997). Due to the higher herbicide input in vegetable and cereal fields (Roßberg et al. 2002; Roßberg 2009b; a) a lower plant diversity was observed in these crops.

Although the Lepidoptera were trapped in agricultural sites in this study semi-natural habitats are essential for numerous arthropod species inhabiting agro-ecosystems (Duelli & Obrist 2003). Moth populations can benefit from an increasing availability of seminatural habitats at a local level (Fuentes-Montemayor et al. 2011). The relevance of field margins as a source of lepidopteran host plants was exemplarily assessed using Noctuidae and their known host plants (Figure 2). Especially field margins bordering cereal fields contained greater numbers of host plants compared to the in-field area of the crop. Thus, field margins can play a significant role in the preservation of the lepidopteran diversity in the agricultural landscape. Therefore, the appropriate management of field margins is of great concern. Especially the time of mowing (Feber et al. 1996) as well as the width of the margins (Welling et al. 1988) could have an influence on the floral diversity. Furthermore, the exposure of field margins to fertilizers and herbicides can lead to declines in plant species richness (Kleijn & Snoeiijing 1997). The resulting reduced availability of host and nectar plants may affect the value of field margins to butterflies (and moths) (Longley & Sotherton 1997).

Differences in the occurrence of nocturnal insects may affect bats. In the following the results of our insect trapping are discussed in respect to the food demands of two bat species, the pipistrelle (*Pipistrellus pipistrellus*) and the grey long-eared bat (*Plecotus austriacus*). Both species are known to prey in agricultural landscapes (e.g. Wickramasinghe et al. 2004; Stahlschmidt & Brühl 2012) and occurred also in our study regions (Stahlschmidt et al.

submitted). While the former one hunts predominantly Diptera, Microlepidoptera and Trichoptera (Eichstädt & Bassus 1995; Wickramasinghe et al. 2004) the later one is specialized in preying on Noctuidae (Bauerova 1982; Vaughan 1997).

In both arable crops Diptera (mostly Nematocera) was the most abundant order while Trichoptera and Microlepidoptera were also available but in smaller numbers (Figure 1). Therefore, arable crops appear to provide suitable food resources for the pipistrelle. In the vineyards, abundances of Diptera were much lower. These results are in accordance with a study by Stahlschmidt et al. (2012) where low prey density as well as low activity levels of the pipistrelle were found. The importance of wetland creation as a compensation measure to provide foraging habitat for bat species preying on small aerial insects in areas where land use is dominated by vineyards was demonstrated (Stahlschmidt et al. 2012).

However, the much higher abundances of Noctuidae over vineyards compared to the arable crops fulfil the food requirements of the grey long-eared bat, an endangered species, whose predominant occurrences at vineyards was also demonstrated in the study region (Stahlschmidt et al. submitted).

Previous research on insect availability in agro-ecosystems focused predominantly on farmland birds and their food demands (Wilson et al. 1999; Holland et al. 2012) but our results indicate that bats could also be affected. A decline of key insect groups like Lepidoptera and Diptera (Vaughan 1997) might be detrimental to bat populations. At least for most Lepidoptera the availability of caterpillar host plants is a fundamental condition for their occurrence. Noctuidae and their specialist predator, the grey long-eared bat, could therefore benefit from the creation of plant species rich strips of (permanent) vegetation within or adjoining to the agricultural sites in combination with a reduced pesticide input (e.g. via agri-environmental schemes).

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Appendix V **Constructed wetlands support bats in agricultural landscapes**

Constructed wetlands support bats in agricultural landscapes

Peter Stahlschmidt, Achim Pätzold, Lisa Ressler, Ralf Schulz & Carsten A. Brühl

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Constructed wetlands support bats in agricultural landscapes

Peter Stahlschmidt*, Achim Pätzold, Lisa Ressler, Ralf Schulz, Carsten A. Brühl

Institute for Environmental Sciences, University of Koblenz-Landau, Fortstr. 7, 76829 Landau, Germany

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Abstract

Bats are known to use aquatic habitats as foraging habitats. Agricultural intensification is perceived to be a main reason for the loss of wetlands. However, artificial wetland creation (i.e. the construction of retention-ponds) in the agricultural landscape aiming at water or nutrient retention has recently gained importance. We evaluated to what extent bats use these artificial wetlands as foraging habitats in an agricultural landscape.

Bat activity and prey density were compared in matched pairs at retention-ponds and neighbouring vineyard sites using stationary bat-detectors and sticky-traps, respectively. To examine if bat activity is related to the number of bat individuals, a thermal infrared imaging camera was used. *Pipistrellus pipistrellus*, the dominant species, served as an example to assess habitat selection between retention-ponds and vineyards. This was performed by relating foraging activity to the available area available within the potential home-range.

Total bat activity and nocturnal prey density were significantly higher above the retention-ponds than above vineyards. High differences of activity levels between the ponds and the respective vineyard sites were found for *Pipistrellus* spp. (*P. pipistrellus* and *P. nathusii*) and *Myotis* spp. (*M. daubentonii* and *M. mystacinus*), being about 180 times and 50 times higher above the retention-ponds, respectively. A significant correlation was found between recorded bat activity and the maximum number of bat individuals observed with a thermal infrared imaging camera. When relating foraging activity to habitat availability within the assumed home-range of *P. pipistrellus*, retention-ponds had on average a higher importance as a foraging habitat than the complete vineyard area although they covered less than 0.1% of its area.

This study indicates that artificial wetlands such as retention-ponds provide foraging habitats for bats. Therefore, creation of wetlands in intensively used agricultural landscapes benefits bats.

Zusammenfassung

Fledermäuse nutzen Gewässer als Nahrungshabitate. Die landwirtschaftliche Intensivierung wird als Hauptgrund für den Verlust an Gewässern angesehen. In dieser Studie wurde untersucht, welche Bedeutung künstlich angelegte Gewässer zum Zweck der Regen- oder Nährstoffrückhaltung in landwirtschaftlichen Flächen als Nahrungshabitate für Fledermäuse haben.

Mit Hilfe von stationären Fledermausdetektoren und Klebefallen wurden sowohl die Fledermausaktivität als auch die Nahrungsdichten der entsprechenden Beuteinsekten parallel an Regenrückhaltebecken und in nahe gelegenen Weinbergen gemessen. Mit Hilfe einer Wärmebildkamera wurde untersucht ob es einen Zusammenhang zwischen Fledermausaktivität und Individuenanzahl gibt. Um die Bedeutung beider Habitattypen als Nahrungshabitat abzuschätzen, wurde am Beispiel von *Pipistrellus pipistrellus* die gemessene Jagdaktivität auf die Flächenanteile beider Habitate innerhalb des Aktionsradius dieser Art bezogen.

*Corresponding author. Tel.: +49 634128031318; fax: +49 634128031326.

E-mail address: stahlschmidt@uni-landau.de (P. Stahlschmidt).

Die Fledermausaktivität und die Dichten nachtaktiver Insekten waren über den Regenrückhaltebecken signifikant höher. Besonders große Unterschiede an Fledermausaktivität wurden für *Pipistrellus* spp. (*P. pipistrellus* und *P. nathusii*) und *Myotis* spp. (*M. daubentonii* und *M. mystacinus*) nachgewiesen, die im Vergleich zu den Weinbergen 180 beziehungsweise 50-mal höher war. Weiterhin wurde eine Korrelation zwischen registrierter Fledermausaktivität und der maximalen Zahl beobachteter Fledermäuse nachgewiesen. Bezieht man die Jagdaktivität auf die Flächenanteile der beiden untersuchten Habitattypen innerhalb des Aktionsradius von *P. pipistrellus* so zeigt sich, dass die Regenrückhaltebecken im Mittel eine höhere Bedeutung als der gesamte Weinbergbereich haben, obwohl sie weniger als 0.1% von dessen Fläche ausmachen.

Künstlich angelegte Gewässer wie z.B. Regenrückhaltebecken stellen wichtige Nahrungshabitats für Fledermäuse dar. Neben dem Nutzen des Wasser- und Nährstoffrückhalts profitieren auch Fledermäuse durch die Anlage von Regenrückhaltebecken in landwirtschaftlich geprägten Gebieten.

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Keywords: Agricultural intensification; Artificial wetlands; Aquatic-terrestrial subsidy; Conservation; Habitat selection; *Myotis*; *Pipistrellus*; Retention-ponds

Introduction

Agricultural intensification has resulted in large-scale changes in the European landscape during the second half of the 20th century. Traditional, less extensive mixed farming systems have been replaced by high input/output systems with homogenous and highly productive arable fields which are generally treated with agrochemicals (Thiere et al. 2009). This has caused major losses in non-crop habitats such as hedgerows, field margins and especially wetlands, which have been lost on a large scale (Amezaga, Santamaria, & Green 2002). Changes in agricultural practice and decrease of landscape heterogeneity have led to an impoverishment of farmland biodiversity (Krebs, Wilson, Bradbury, & Siriwardena 1999). In countries where agricultural intensification has already resulted in an alarming level of ecological degradation, more and more efforts are recognisable to improve landscape heterogeneity with ecological compensation programs. In this respect, wetland creation in the agricultural landscape (e.g. EU Life project ArtWET) with the aim to improve ecosystem services like nutrient, pesticide and water retention (e.g. Stehle et al. 2011) and biodiversity (Thiere et al. 2009) has recently received much attention. Wetlands are of particular importance for biodiversity conservation, because they provide refuges and food for aquatic, amphibian, and terrestrial animals (Mitsch & Gosselink 2000).

Many bat species are known to use aquatic ecosystems as foraging habitats (e.g. Vaughan, Jones, & Harris 1997; Scott, McLaren, Jones, & Harris 2010). Recent studies have demonstrated that in natural ecosystems emerging aquatic insects can form an important food resource for bats (Fukui, Murakami, Nakano, & Aoi 2006; Hagen & Sabo 2011). But not only natural wetlands are known as foraging habitats, also artificial systems such as sewage treatment works are used by certain bat species (Park & Christinacce 2006). Given that the decline of insects associated with the loss of habitat due to agricultural intensification is a major cause for the observed declines in many European bat populations during the second half of the 20th century (Stebbing 1988), we need to

better understand if the creation of wetlands can compensate for some of those losses by providing islands of higher prey availability in agricultural landscapes.

In the present study we examined the role of artificial retention-ponds as foraging habitats for bats in an intensively managed agricultural landscape (vineyards). We predicted that the availability of flying insects and, consequently, the foraging activity of bats is higher above the ponds than in the surrounding vineyards.

Materials and methods

Study sites and sampling

The study was conducted in the wine-growing region of Rhineland-Palatinate, SW Germany, around Landau (49°7′–49°18′N, 8°02′–8°09′E). The climate of the region is characterised by an average annual temperature of 10 °C and an annual precipitation rate of about 600 mm. Sampling was established at seven retention-ponds (sites A–G) surrounded exclusively by vineyards. Retention-pond sizes ranged from 0.1 to 1.3 ha (see Appendix A). As bats are known to prefer water habitats bordered by a bank-side vegetation (e.g. Warren, Waters, Altringham, & Bullock 2000; Scott et al. 2010) we only selected retention-ponds with 85% or more of the bank line covered with bushes or trees.

At each site, bat activity and nocturnal insect availability was assessed simultaneously at the retention-pond and in a corresponding vineyard site (approximately 80 m away from the pond edge). To consider temporal variability each site-pair was surveyed 8–9 times (8 sampling nights for three site-pairs B, D and F; 9 sampling nights for four site-pairs A, C, E and G), resulting in a total of 120 sampling nights. We sampled only during nights when weather conditions were favourable for bat activity (Kusch, Weber, Idelberger, & Koob 2004), i.e., no rain, wind speed below 10 km/h, and temperatures above 17 °C at sunset. The study was conducted from the end of June until the end of August 2009, coinciding with the lactation period of most European bats (Vaughan et al. 1997).

Table 1. The classification of the species groups used in the statistical analysis.

Species group	Species actually recorded	bcDiscriminator group ^a
' <i>Pipistrellus nat-pip</i> '	<i>Pipistrellus nathusii</i> <i>Pipistrellus pipistrellus</i>	'Pipistrelloid'
' <i>Myotis dau-mys</i> '	<i>Myotis daubentonii</i> <i>Myotis mystacinus</i>	'Mkm'
' <i>Myotis myo-nat</i> '	<i>Myotis myotis</i> <i>Myotis nattereri</i>	'Mmyo' 'Mnat'
' <i>Eptesicus-Nyctalus</i> '	<i>Eptesicus nilssonii</i> <i>Eptesicus serotinus</i> <i>Nyctalus leisleri</i> <i>Nyctalus noctula</i>	'Nyctaloid'

^aThe species groups 'Pipistrelloid', 'Mkm' and 'Nyctaloid' contain additional species not recorded in the present study.

Bat activity

We recorded bat activity using automatic stationary bat detector systems, so-called 'batcorders' (ecoObs GmbH, Nürnberg, Germany). Batcorders were installed at a height of 3.5 m above the ground and adjusted to the system's standard settings (Runkel 2008). The sampling points were chosen in a way that assured uncluttered acoustic space within the detection radius of the system (10 m; Runkel 2008). At the ponds, batcorders were installed 2 m inland from the water's edge to avoid interference caused by close runtimes of calls and their echoes of bats flying just above the water surface. The recording was done with one batcorder at each site from sunset to sunrise.

Bat activity was measured as seconds of recorded call-sequences per night. We analysed call-sequences using bcDiscriminator (ecoObs GmbH, Nürnberg, Germany), an automatic identification software which gradually determines call-sequences to species groups and then, if possible, to species. In some cases it is difficult or impossible to distinguish between species due to similarities in call structure and only the assignment to a species group with similar acoustic repertoires is possible. In order to perform statistical analyses we used only species groups (see Table 1 for definition of species groups). For every determination bcDiscriminator reports a degree of confidence. We subsequently analysed call-sequences with an identification confidence below 80% for the corresponding bcDiscriminator species group (Table 1) with the software bcAnalyze (ecoObs GmbH, Nürnberg, Germany) and either assigned them to a group or classified them as 'unidentified'.

Acoustic measures of bat activity do not allow direct conclusions about the number of individuals. To estimate the role of foraging habitats for bats, it is also important to know if higher activity levels are caused by higher number of individuals or, as an extreme case, by a single individual which is just very frequently recorded due to the restricted habitat

size. We therefore evaluated the relation between recorded bat activity and the number of bats by using a thermal infrared imaging camera (VarioCam, Infra Tec, Dresden, Germany). To standardise the survey by having comparable observation fields, the camera was focused on the steel pole of the batcorder (3.5 m high) erected approximately 35 m away (in the case of retention-ponds at the opposite bank site). As a measure of bat individuals we considered the maximum numbers of bats seen simultaneously in the observation field within a time interval of 1 h. The retention-pond sites A and D were surveyed on four, and the sites B, C, E, F and G on three different nights. Two vineyard sites (site D and F) were only observed once.

Food availability

Parallel to the bat recording, we measured nocturnal activity density of aerial insects using sticky-traps. At each retention-pond, we established a line in North-South direction about 2 m above the water surface of the ponds and another line about 2 m above the ground in an adjacent vineyard. Fourteen sticky-traps were attached to each line (seven facing East and seven facing West). Each trap consisted of a clear plastic sheet (15.0 cm × 23.7 cm) covered with Tangle-Trap glue (Tanglefoot, Grand Rapids, Michigan, USA). To assure that only nocturnal insects were sampled, sticky-traps were set up at sunset and collected at sunrise. Insects were measured (±1 mm) and grouped in size classes to investigate the relationship between the activity of the bat groups and the abundance of their prey items at preferred size ranges. The prey size suitable for '*Pipistrellus nat-pip*' is reported to be around 3 mm on average (Barlow 1997) and mainly less than 5 mm (Beck 1995). Thus, the main prey size was considered to be 2–5 mm. According to Taake (1992), *M. daubentonii* and *M. mystacinus* ('*Myotis dau-mys*') feed mainly on insects in the range of 3–10 mm. *M. myotis* and *M. nattereri* ('*Myotis myo-nat*') are not aerial hunters and their prey could not be assessed by the applied insect trapping method. *M. myotis* feeds almost exclusively on carabid beetles (Beck 1995) and *M. nattereri* mainly gleans arthropods from the vegetation (Vaughan 1997). Regarding prey abundance, species grouped under '*Eptesicus-Nyctalus*' were also excluded from further analysis, since the diet of these species varies remarkably with regards to composition and preferred prey size ranges (Krapp 2004 and references therein).

We identified insects to order, Diptera to sub-order (Brachycera) or family (Nematocera) and categorised their larval origin as aquatic or terrestrial. The larval stages of Coleoptera, Brachycera and Tipulidae and Limoniidae (Nematocera) are either aquatic or terrestrial, thus, they were listed as 'of unknown origin'. Chironomidae and Ceratopogonidae were grouped as 'of aquatic origin' as there is only a negligible number of species with terrestrial larval stages.

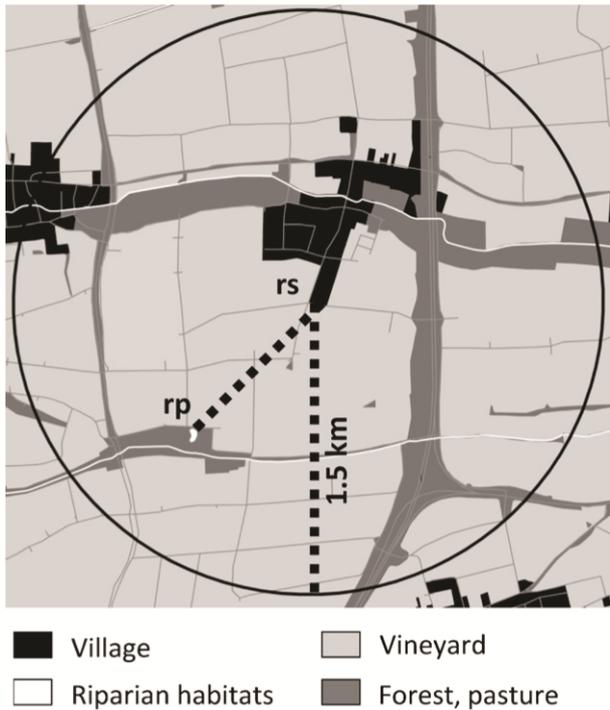


Fig. 1. Schematic drawing of a representative study site (Site C) with the retention-pond (rp) and the nearest housing of the closest village as a potential roost site (rs). The area enclosed by the circle with a radius of 1.5 km indicates the approximate home-range of *Pipistrellus pipistrellus*.

Habitat use versus habitat availability

Pipistrellus pipistrellus as the most common bat species in the study was used to evaluate the general importance of retention-ponds as foraging habitats compared to vineyards. Therefore, we related the calculated foraging activity to the coverage of both habitats within the assumed home-range of *P. pipistrellus*. As this species is known to prefer buildings as roost sites, the nearest building of the village closest to each retention-pond was assumed as the potential roost site (Fig. 1). The mean distance between roost and foraging areas for this species has been recorded as 1.5 km (Davidson-Watts & Jones 2006). We determined the area coverage of land-use types within a 1.5 km radius from those potential roost sites using ArcView GIS 9.3 (ESRI, Redlands, USA) and land-use information (ATKIS 2008, AdV, Germany) (Fig. 1).

We assessed foraging activity for *P. pipistrellus* by considering feeding buzz rates. A feeding buzz is a call-sequence that becomes shorter in duration and broader in bandwidth in order to provide the bat with information on the position of a prey immediately before capture (Griffin, Webster, & Michael 1960) and therefore acts as an indicator of foraging activity. We analysed every tenth call-sequence of *P. pipistrellus* of every site and night (corresponding to 6830 calls). For every site the number of feeding buzzes were counted, summed up and divided by the total duration of the examined call-sequences and termed as the feeding buzz rate (feeding

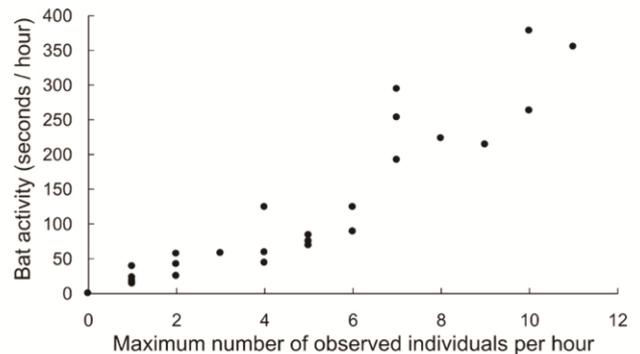


Fig. 2. Scatter plots of measured bat activity against maximum number of bats observed simultaneously by means of a thermal infrared imaging camera within 1 h.

buzzes per second). The site-specific foraging activity was quantified by multiplying the corresponding recorded mean activity and the site-specific feeding buzz rate.

Statistical analysis

Statistical analyses were conducted using SPSS ver. 17 (SPSS, Chicago, USA). The differences in bat activities and insect densities between both habitats at the different sites were analysed using paired *t*-tests. Spearman's correlation coefficient was used to explore the relationship between recorded bat activity and the maximum numbers of observed bats.

Results

Bat activity

In 120 sampling nights a total of more than 75,000 call-sequences were recorded. This corresponds to about 25 h of recorded bat activity of which 98.3% was recorded above the retention-ponds and 1.7% above the vineyards. About 92% of the recorded bat activity time was assigned to '*Pipistrellus nat-pip*' (almost 94% were identified as *P. pipistrellus*), 3.0% to '*Eptesicus-Nyctalus*', 4.4% to *Myotis* (92% were identified as '*Myotis dau-mys*', about 5.0% as '*Myotis myo-nat*' and less than 3% as 'unidentified *Myotis*'), and 0.6% were classified as 'unidentified'.

Total bat activity (all species) was significantly higher above the retention-ponds than above the respective vineyard sites (Table 2). When bat groups were analysed separately, significantly higher activity levels above retention ponds were found for all groups with the exception of '*Eptesicus-Nyctalus*' (Table 2).

A significant positive correlation between bat activity and the maximum number of bats observed by means of a thermal infrared imaging camera was found ($r_s = 0.965$, $P < 0.001$, $n = 26$; Fig. 2).

Table 2. Statistical comparison between bat activities (seconds per night) and insect densities (number of insects of 14 sticky-traps per night) of paired retention-ponds (Rp) and vineyards (Vi). The values for Rp and Vi represent the mean (and standard deviation) of the means of seven sites, each of them measured 8–9 times, *t* and *P*-values for paired *t*-tests are given. Significant *P*-values (*P* < 0.05) are in bold.

	Rp (SD)	Vi (SD)	<i>n</i>	<i>t</i>	<i>P</i>
<i>Bat activity</i>					
Bats (total)	1543.4 (1632.9)	25.1 (11.3)	7	2.5	0.048
' <i>Pipistrellus nat-pip</i> '	1420.8 (1513.4)	7.7 (2.6)	7	2.5	0.048
' <i>Myotis dau-mys</i> '	62.2 (48.8)	1.2 (0.9)	7	3.3	0.016
' <i>Myotis myo-nat</i> '	2.9 (1.9)	1.6 (1.4)	7	2.8	0.030
' <i>Eptesicus-Nyctalus</i> '	55.4 (67.8)	14.3 (6.1)	7	1.6	0.161
<i>Insect density</i>					
Total insect density	82.0 (45.3)	23.8 (8.2)	7	3.4	0.014
Prey of ' <i>Pipistrellus nat-pip</i> '	74.3 (42.9)	20.9 (6.6)	7	3.4	0.014
Prey of ' <i>Myotis dau-mys</i> '	51.1 (26.4)	13.3 (4.3)	7	3.9	0.008
Aquatic origin	57.2 (41.7)	2.8 (1.2)	7	3.5	0.013
Terrestrial origin	19.2 (6.9)	16.5 (5.3)	7	1.3	0.254
'Unknown' origin	5.6 (4.8)	4.5 (3.1)	7	0.8	0.467

Food availability

In total 5978 insects with a body size > 2 mm were trapped in 120 sampling nights; 76.9% of all insects were trapped at the retention-ponds and 23.1% in the neighbouring vineyards. Total density of nocturnal insects and densities in the size ranges of 2–5 mm and 3–10 mm, suitable prey for '*Pipistrellus nat-pip*' and '*Myotis dau-mys*', respectively, were significantly higher above the ponds (Table 2).

The total of 4595 insects trapped above the retention-ponds consisted of 71% of emerged aquatic insects, 23% of insects with terrestrial larval stages and 6% of 'unknown origin'. Of the 1381 insects recorded from the vineyards 69% had a terrestrial, 12% an aquatic and 19% an 'unknown origin'. Densities of insects with aquatic larval stages were significantly and on average almost 21 times higher above the retention-ponds (Table 2). There were no significant differences between ponds and vineyards for insects with terrestrial or 'unknown' larval stages (Table 2).

Habitat use versus habitat availability

Feeding buzz rates of on average 0.234 and 0.028 feeding buzzes per second for *P. pipistrellus* were found for the retention-pond and vineyard sites, respectively. The resulting foraging activities for each site are shown in Table 3. The foraging activity relative to the area coverage of the respective habitat indicated that the examined retention-ponds, although covering less than 0.1% (see Appendix A) of the non-settlement area within the home-range of *P. pipistrellus*, had on average a higher importance as foraging habitats than the vineyards (Table 3) which covered on average 81.6% (see Appendix A). The retention-pond sites A, B and D had even greater importance as foraging habitats than the entire vineyard area within the home-range (Table 3).

Discussion

Freshwater habitats have been found to be valuable foraging habitats for bats (e.g. Vaughan et al. 1997; Grindal, Morissette, & Brigham 1999; Russo & Jones 2003; Scott et al. 2010). Vaughan et al. (1997) emphasised the need to know which types of freshwater habitats are preferred by bats, and how they can be managed to attract more bats. In general, the way in which wildlife cope with new environments in an anthropogenically modified and transformed world is of importance for biodiversity managements (Pimm & Gittleman 1992). In the present study, we have demonstrated that retention-ponds in a landscape dominated by extensive wine-growing were used by bats as foraging habitats. In Europe about 3% of the agricultural land is composed of vineyards (European Commission 2008) but in wine-growing areas such as Southern Palatinat or Bordeaux vineyards are in general the exclusive land use and dominate the landscape.

With the construction of retention-ponds, islands of higher prey availability can be created in agricultural landscapes where declines in insect abundance associated with agricultural intensification have been suggested as a main factor for the avoidance of bats (Wickramasinghe, Harris, Jones, & Vaughan 2003) and the decrease of bat populations (Stebbins 1988).

By using a thermal infrared imaging camera, we demonstrated that activity levels at the retention-ponds were associated with the number of bat individuals. This indicated that the ponds were not only foraging sites for a single or a few individuals causing high numbers of recorded call-sequences due to the restricted habitat space, but that the retention-ponds did indeed attract more bat individuals.

The importance of these constructed habitats becomes even more evident when taking into account the ratios of foraging activities and habitat availability within a species home-range (Fig. 1 and Table 3). A relatively small retention-pond can be of higher value as a foraging area than the predominant

Table 3. Comparison of foraging activity of *Pipistrellus pipistrellus* on retention-ponds (Rp) and vineyards (Vi) and the availability of both habitats. Area coverage of retention-ponds and vineyard refer to an assumed 1.5 km home-range distance of *P. pipistrellus* measured from the nearest settlement of the respective retention pond (sites A–G). Values of activity of *P. pipistrellus* shown are the means of 8–9 sampling nights. Foraging activity was calculated as the product of measured mean activity of each site and the respective feeding buzz rate.

	A	B	C	D	E	F	G	Mean (SD)
<i>Retention-ponds</i>								
Activity ^a	4345.5	1378.1	1431.8	1172.6	453.1	343.7	332.1	1380.7 (1538.0)
Feeding buzz rate	0.269	0.223	0.254	0.230	0.221	0.213	0.227	0.234 (0.022)
Foraging activity (f_{Rp})	1168.9	307.3	363.7	296.7	100.1	73.2	75.4	336.9 (421.2)
Area ^b (a_{Rp})	0.39	1.34	0.11	0.28	0.26	0.13	0.11	0.37 (0.48)
Area activity ($f_{Rp} \times a_{Rp}$)	455.9	411.8	40.0	75.5	26.0	9.5	8.3	146.7 (197.9)
<i>Vineyards</i>								
Activity ^a	10.6	8.8	6.8	4.1	10.1	5.6	4.1	7.2 (2.7)
Feeding buzz rate	0.031	0.048	0.023	0.026	0.039	0.019	0.020	0.028 (0.011)
Foraging activity (f_{Vi})	0.33	0.42	0.16	0.11	0.39	0.10	0.08	0.22 (0.15)
Area ^b (a_{Vi})	544.2	440.5	467.2	398.1	407.1	389.9	469.1	445.2 (54.2)
Area activity ($f_{Vi} \times a_{Vi}$)	179.6	185.0	74.8	43.8	158.8	38.9	37.5	101.0 (68.8)
<i>Ratio of area activities</i>								
(Rp/Vi)	2.5	2.2	0.5	1.7	0.2	0.3	0.2	1.1 (1.0)

^aIn seconds per night.

^bIn ha.

habitat (e.g. vineyards), although covering less than 0.1% of its area. Although other species may have much larger home-ranges than *P. pipistrellus* and are able to reach productive natural foraging habitats, they are likely to benefit in a similar way from reliable foraging areas close to their roosts. It has been demonstrated that especially for lactating females, the cost of commuting is important since percentage of low-weight young and offspring mortality are closely related to the distance travelled by the female bats to their feeding areas (Tuttle 1976).

Differences in bat activities between the two habitat types were especially high for ‘*Pipistrellus nat-pip*’ (*P. nathusii*, *P. pipistrellus*) and ‘*Myotis dau-mys*’ (*M. daubentonii*, *M. mystacinus*). For both bat groups, higher availability of appropriate prey-insect densities were also found, demonstrating that bats selected retention-ponds as foraging habitats. An additional reason for the preferential use of the examined ponds for foraging might be the bankside vegetation. The presence of a vegetation edge concentrates insects (Verboom & Spoelstra 1999) and allows bats to forage earlier in the evening, because it suppresses light intensity that can lead to higher risk of predation by owls (Rydell, Entwistle, & Racey 1996).

Although mainly feeding on arthropods with terrestrial origin, a significantly higher activity of the group ‘*Myotis myo-nat*’ (*M. myotis*, *M. nattereri*) was found at the retention-ponds. Most of the recorded call-sequences belonged to *M. nattereri* (data not shown) which has probably benefited from higher arthropod densities in the riparian vegetation. Arthropods, such as spiders, can occur in higher densities close to aquatic habitats due to the higher prey availability by emerged aquatic insects (Paetzold, Schubert, & Tockner 2005).

Due to the problems of determining short call-sequences and the differences in prey-preferences of species compiled as ‘*Eptesicus-Nyctalus*’, this group was excluded from evaluations regarding prey availabilities. However, with the exception of *Eptesicus serotinus* (mainly preying on beetles), the other members assigned to that group, *Nyctalus leisleri*, *N. noctula* and *Eptesicus nilssonii*, are known to feed on nematoceran Diptera with aquatic origin (Beck 1995; Vaughan 1997). Although no significant differences between both habitats have been found for ‘*Eptesicus-Nyctalus*’ in general, they are likely to benefit from the higher insect availability above retention-ponds. At retention-pond site B, *E. nilssonii* was recorded regularly (data not shown), a species reported locally as facing extinction (König & Wissing 2007).

Apart from the recorded species in our study, other bat species such as *P. pygmaeus*, *M. brandtii*, *M. dasycneme*, *Vespertilio murinus* and *Rhinolophus hipposideros* are likely to gain from the construction of artificial wetlands as insects of aquatic origin represent major parts of their diet (Rydell 1992; Vaughan 1997). And although *P. pipistrellus*, the most common bat species in our study, remains the most abundant and widespread bat species in Europe, estimates from the Annual Bat Colony Survey in the UK suggested a decline of over 60% between 1978 and 1993 (Hutson 1993). Thus, by representing important local foraging patches, artificial wetlands in the agricultural landscape may benefit a considerable number of bat species, among them highly endangered ones. Our results support the conclusion of Duelli and Obrist (2003) that the conservation and creation of natural and seminatural habitat ‘islands’ is the most promising way to enhance or restore species richness in agricultural landscapes and we like to emphasise the importance of wetland habitats.

Aquatic to terrestrial exchange of aquatic-derived energy, nutrients and organism have been shown to have profound effects on the distribution of terrestrial consumers in general (e.g. Power & Rainey 2000; Paetzold et al. 2005) and on bats in particular (Fukui et al. 2006; Hagen & Sabo 2011). Our results suggest that the creation of ponds in the agricultural landscape, although small in size, can have strong positive effects on the food resource of bats roosting in areas dominated by agriculture. This may have also consequences on their distribution and population size.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.baae.2012.02.001.

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Appendix A. Area coverage of the retention-ponds and the non-settlement land use types (vineyard, other arable land, forest and pasture) within the assumed home-range of *Pipistrellus pipistrellus* at the sites A-G. Area coverage refers to an assumed 1.5 km home-range distance of *P. pipistrellus* measured from the building (potential roost site) closest to the respective retention-pond (see Fig. 1).

		A	B	C	D	E	F	G	Mean
Retention-pond	(ha)	0.4	1.3	0.1	0.3	0.3	0.1	0.1	0.4
	(%)	< 0.1	0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Vineyard	(ha)	544.2	440.5	467.2	398.1	407.1	389.9	469.1	445.2
	(%)	87.9	76.2	93.9	80.0	90.4	70.9	71.7	81.6
Arable land	(ha)	14.5	44.1	1.1	11.8	1.0	95.1	104.9	38.9
	(%)	2.3	7.6	0.2	2.4	0.2	17.3	16.0	6.6
Forest, pasture	(ha)	60.3	92.1	29.2	87.5	41.8	64.5	80.6	65.1
	(%)	9.7	15.9	5.9	17.6	9.3	11.7	12.3	11.8

Appendix VI Bats at risk? Bat activity and insecticide residue analysis of food items in an apple orchard.

Bats at risk? Bat activity and insecticide residue analysis of food items in an apple orchard

Peter Stahlschmidt & Carsten A. Brühl

Environmental Toxicology and Chemistry, 31(7): 1556-1563



BATS AT RISK? BAT ACTIVITY AND INSECTICIDE RESIDUE ANALYSIS OF FOOD ITEMS IN AN APPLE ORCHARD

PETER STAHLSCHEMIDT and CARSTEN A. BRÜHL*

Institute for Environmental Sciences, University of Koblenz-Landau, Landau, Germany

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Abstract—Although bats are reported as being threatened by pesticides, they are currently not considered in European Union pesticide risk assessments. The reason for that contradiction is probably related to the scarcity of information on bat activity in pesticide-treated fields and the pesticide residues on their food items. The authors recorded bat activity and measured pesticide residues on bat-specific food items following applications of two insecticides in an apple orchard. High activity levels of the common pipistrelle bat, a foraging habitat generalist, were detected. Airborne foragers and bats that take part of their food by gleaning arthropods from the vegetation were recorded frequently. The initial value and the decline of pesticide residues were found to depend on the arthropod type, their surface to volume ratio, their mobility, and the mode of action of the applied pesticide. The highest initial residue values were measured on foliage-dwelling arthropods. By following the toxicity-exposure ratio approaches of the current pesticide risk assessment, no acute dietary risk was found for all recorded bat species. However, a potential reproductive risk for bat species that include foliage-dwelling arthropods in their diet was indicated. The results emphasize the importance of adequately evaluating the risks of pesticides to bats, which, compared to other mammals, are potentially more sensitive due to their ecological traits. *Environ. Toxicol. Chem.* 2012;31:1556–1563. © 2012 SETAC

Keywords—Chlorpyrifos-methyl Fenoxycab Mammal Risk assessment Sensitivity

INTRODUCTION

The European Union first-tier assessments of acute and reproductive risk of pesticides to birds and mammals [1] are based on toxicity-exposure ratios (TERs), which are compared to safety factors (trigger values). If the TER is larger than the safety factor, the risk is considered to be low. If the TER is lower than the safety factor, no authorization is granted for the pesticide unless a refined risk assessment demonstrates that no risk for wildlife species occurs when the pesticide is applied under field conditions. For the toxicity component of the ratio, the LD50 (lethal dose, the dose at which 50% of the test organisms die) of an acute oral test for birds and for mammals is used for the acute risk assessment, whereas the no observed adverse effect level (NOAEL) values of reproduction tests (birds) and of multigeneration studies (mammals) are used for the reproductive risk assessment. Dietary exposure is estimated by applying a number of different generic indicators (“generic focal species”), which are not real species, however, regarding their feeding habits, representative for species that occur in a particular crop at a particular time. Based on the food-intake rate, the body mass, and the concentration of the compound in the diet, shortcut values of these generic indicators are available for a range of scenarios (type of crop, growth stage of the crop, and kind of application) [1].

Insectivorous mammals are represented by the generic indicator “shrews” but no reference is made to bats, a group of 42 species comprising one-fifth of all European terrestrial mammals [2], differing widely in feeding habits from shrews because they hunt flying insects and feed on arthropods on the vegetation (gleaning). Therefore, potential dietary exposure to pesticides is

different. Considering that generic focal species should be representative for all species that could be at risk [1], bats are obviously not supposed to be exposed to pesticides. Controversially, the agreement on the conservation of European bat populations [3] stated in article III (fundamental obligations), number 8: “Each party shall, wherever appropriate, consider the potential effects of pesticides on bats, when assessing pesticides for use. . . .”

Evidence of pesticide exposure of bats was discovered in the 1960s and 1970s, a period of widespread use of organochlorine pesticides. Some of these pesticides were responsible for the significant mortality of several bat species as demonstrated by field and laboratory studies in northern America and Europe [4–6]. A die-off of juvenile greater mouse-eared bats (*Myotis myotis*) was documented after the application of methamidophos (Filitox, an organophosphate) to nearby potato fields and apple orchards in Germany [7]. The high levels of methamidophos residues detected in the corpses were considered to be transferred through milk to the offspring by females that consumed contaminated insects. In Spain, residues of fenitrothion (organophosphate) were reported in common pipistrelles (*Pipistrellus pipistrellus*) following agricultural applications [8]. Today, most highly toxic and persistent pesticides have been replaced; therefore, the effects of modern pesticides on bats may be more difficult to document, have been less well studied, and are probably underestimated [9].

Apart from the direct evidence of exposure, recent radio-tracking studies and acoustic surveys performed with bat detectors revealed high foraging activity of bats in different kinds of orchard crops in Europe. Intensively managed apple orchards were documented as being positively selected as foraging habitats by the greater mouse-eared bat in southwestern Switzerland [10] and Tyrol [11]. Foraging activity of bats was also reported in intensively cultivated olive orchards treated with insecticides in Greece [12]. Data on foraging

* To whom correspondence may be addressed
(bruehl@uni-landau.de).

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activity of bats in other agricultural crop fields are scarce and do not allow a profound conclusion. For example, Walsh and Harris [13] found that arable land in Britain was avoided by bats, while Russo and Jones [14] recorded relatively large numbers of foraging attempts in some arable fields in a survey in southern Italy. However, none of these studies give details about the crop and, hence, preclude a consistent conclusion of potential pesticide exposure.

The aim of the present study was to estimate the exposure of bats to pesticides in a conventionally cultivated apple orchard. Bat activity was recorded after two consecutive applications of fenoxycarb (Insegar, a carbamate), an insecticide that is applied up to three times from May to July, which falls in the pregnancy and lactation period of bats. To compare activity levels recorded in orchards with those in habitats known to be used by bats for foraging, we also recorded activity levels in nearby meadow, forest, and forest-edge sites. In parallel, we measured residues of fenoxycarb on the typical food items of the recorded bat species to assess acute and reproductive risk from dietary exposure for the respective bat species. To determine if the mode of action of pesticides influences the residue pattern on arthropods, we additionally measured the residue of chlorpyrifos-methyl (organophosphate) of one arthropod group (foliage-dwelling arthropods) following an application of Reldan in the same orchard.

MATERIALS AND METHODS

Study site and insecticide applications

The present study was conducted in May and July 2009 in a mature commercial apple orchard (Braeburn variety) situated in a fruit-growing area near Winden, Rhineland-Palatinate, southwest Germany ($\sim 49^{\circ}05'N$, $8^{\circ}07'E$). The approximate size of the orchard was 4 ha (160×250 m), consisting of 54 rows of apple trees, with approximately 300 trees, 3.5 m high, in each row. The distance between the rows was approximately 3 m. At both ends of the rows, tractor-turning areas of approximately 10 m widths, covered with lawn and surrounded by apple tree rows, were present (Fig. 1). The apple orchard was surrounded by other conventional apple orchards (north and west), an organic vegetable field (south), and a maize field (east) (Fig. 1). The closest housing of the nearby village of Winden was 400 m away.

The entire 4 ha of the orchard were sprayed with Reldan (Dow AgroSciences) at a rate of $337 \text{ g a.i. ha}^{-1}$ against the woolly apple aphid (*Eriosoma lanigerum*) on one occasion (May 20, 2009) and with Insegar (Syngenta Agro) against the codling moth (*Cydia pomonella*) at $150 \text{ g a.i. ha}^{-1}$ on two occasions (July 1 and 15, 2009). The rates applied represent the recommended application rates in apple orchards according to good agricultural practice. The Reldan application took place after petal fall, and Insegar was applied during the development of fruits. Solutions were prepared on site immediately before application. Both insecticides were applied with a tractor-mounted, air-assisted sprayer (Vicar compact 1200). Two tank mixes (each of 1,200 L) were prepared and applied on each application date. The sprayer was calibrated to deliver 600 L ha^{-1} and configured to produce a spray that reached the highest and lowest branches, while application onto the soil or over the trees was minimized. Applications started at approximately 4:00 PM and lasted approximately 4 h. Spray deposit distribution was documented using water-sensitive papers placed in various positions within the tree canopy and indicated an even spray deposit.

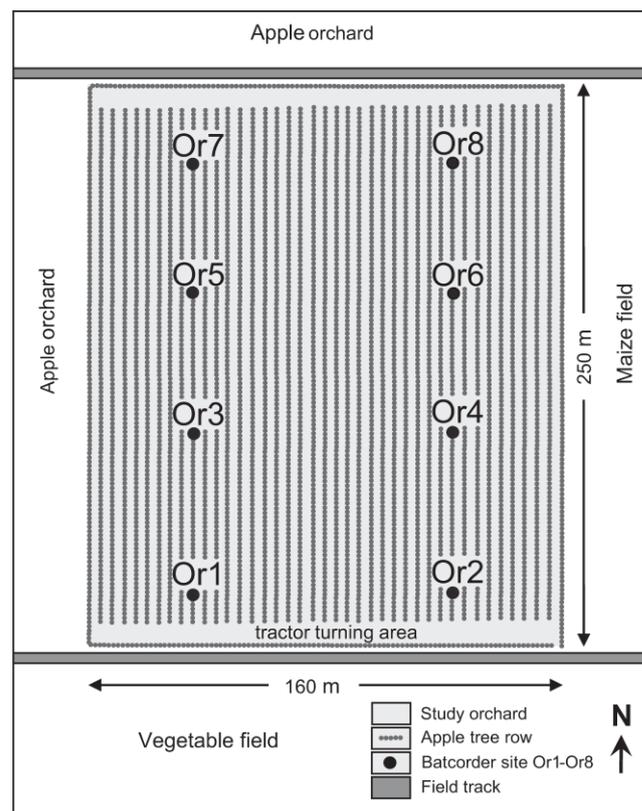


Fig. 1. Schematic diagram of the study site at Winden, Rhineland-Palatinate, Germany.

Bat activity measurement

Acoustic measurement of bat activity is a reliable estimate of foraging activity [14]. We recorded bat activity using several simultaneously working automatic stationary bat-detector systems (batcorder; ecoObs), a method suitable to address spatial and temporal variation in bat activity patterns [15]. Eight batcorders were installed at a height of 1.5 m above the orchard canopy to avoid absorption and reflection of the echolocation calls at eight sites in the orchard, each site with at least a 25 m buffer to the border. Two batcorders (sites Or1 and Or2) were located close to a tractor-turning area, which was surrounded by rows of apple trees on three sides and bordered by apple tree rows on one side (Fig. 1). That area was treated with pesticide in the same way as the remaining area of the orchard. Acoustic recording of bats was only possible during nights without rain and with low wind speed and, therefore, limited to the nights of days 0 (day of application), 1, 2, 3, and 8 following the first Insegar application and of days 0, 2, 3, and 4 following the second Insegar application.

To compare the recorded activity levels of the examined orchard to activity levels of habitats known to be used for foraging, we also measured bat activity at two meadows, two forest-edge sites, and two sites within that deciduous forest at three occasions in 2009 (20.5, 18.6, 12.8). All these sites were located less than 1.5 km away from the village of Winden, assuring that they were, as well as the orchard, within the home range of bats having their roost sites in the closest settlement (Winden). The distance is based on the foraging range of the common pipistrelle (*P. pipistrellus*), the species with the shortest average distance (1.5 km) between foraging and roost sites among the occurring bat species [16,17].

Batorders were adjusted to the system's standard settings [15]. Recordings were made from sunset to sunrise. Bat activity was measured as seconds of recorded call sequences per night. The software packages bcDiscriminator and bcAnalyse (ecoObs) were used to identify the calls to species level whenever possible. Due to the variation in species-specific call structure and interspecific overlapping between acoustic repertoires of *Eptesicus serotinus*, *Nyctalus leisleri*, and *Nyctalus noctula*, it was impossible to assign short call sequences to one of those species with sufficient confidence. Hence, species of that group were assigned to the group "*Nyctalus-Eptesicus*." For the same reason, calls of *Myotis mystacinus* and *Myotis nattereri* were assigned to the group "*Myotis mys-nat*."

Arthropod sampling for residue analysis

Insecticides were applied to the entire 4 ha of the orchard, but the sampling area was restricted to the central part with at least a 25 m buffer to every side. The following three sampling methods were used to collect nocturnal arthropods according to the preferences of the different bat guilds: unattended light trap sampling for large moths (e.g., Noctuidae, Geometridae), light trap sampling for small flying insects (e.g., Diptera, Microlepidoptera), and inventory sampling for foliage-dwelling arthropods (e.g., Arachnida, Hemiptera, Coleoptera). Sampling was performed after dusk to ensure that only arthropods available for bats were collected with the exception of the nights following the insecticide applications, when we did not start sampling before the pesticide film on the apple trees dried to avoid contamination. Light traps were installed at a height of 1.80 m. Thereby, insect attraction was restricted by the rows of apple trees to avoid sampling of insects from outside the apple orchard.

Large moths (body size between 10–20 mm) were sampled with two unattended light traps with two ultraviolet fluorescent tubes (bioform light trap; bioform). The attached buckets were filled with cardboard egg box material and arranged for the moths to settle on until they were collected. To collect small flying insects (mainly Diptera and small moths with body size between 3–10 mm), a light-tower (Müller light-tower; bioform) was used. Insects that were attracted to the light were collected from the surface using a handheld vacuum cleaner to which a nylon-collecting bag was attached. Foliage-dwelling arthropods (insects and spiders) were sampled by beating the apple trees with a stick while holding a beating tray (Dynat tray; bioform) under the area being beaten. To prevent residue contamination of subsequent samples, the beating tray was completely covered by a disposable plastic sheet. During the entire sampling period, disposable material (plastic jars, egg box material, nylon collecting bags, etc.) was changed and the collecting gear wiped with acetone after every sampling event to avoid cross-contamination.

All arthropod samples were collected into plastic jars using forceps and immediately placed in a cooler filled with ice to avoid desiccation of the arthropods and decrease of residue. Samples were stored in a freezer (−20°C) until residue analysis. Temperature during transit and storage was monitored using a calibrated temperature data logger. Small flying insects were sorted on ice to small moths and other small-flying insects. Due to the high number of moth scales remaining in the nylon-collecting bag, the collecting bags were kept for analysis as well. The complete sampling program was performed for both Insegar applications on the nights of day 1 (before application), day 0 (day of application), and days 1, 2, 3, 4, 8, and 12 postapplication. The arthropod sampling after the Reldan appli-

cation comprised the collecting of foliage-dwelling arthropods on the nights of days 0 and 8 postapplication.

Residue analysis

The active substances of Reldan and Insegar are chlorpyrifos-methyl (organophosphate) and fenoxycarb (carbamate), respectively. Analysis of insecticide residue was performed using a modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) method [18]. Each invertebrate sample (~1 g fresh wt) was homogenized in a 50-ml vial filled with 10 ml of acetonitrile. The nylon bags containing moth scales were also placed in vials filled with 10 ml of acetonitrile. After adding 0.5 g NaCl and 0.5 g MgSO₄, the samples were shaken for 20 min and subsequently centrifuged for 5 min at 3,000 rev min^{−1}. A filtrated 1.5-ml aliquot of the supernatant of each sample was employed for analysis with an Agilent 1100 HPLC instrument coupled to an API 4000 Qtrap MS/MS (Applied Biosystems). Different concentrations (0.5, 1, 5, 10, 15, 20, 40, 60, 80, 100 ng ml^{−1}) of analytical standards of chlorpyrifos-methyl and fenoxycarb (Sigma-Aldrich) were analyzed under the same instrumental conditions. Quantification was accomplished using the calibration curve constructed by the absolute amount of chlorpyrifos-methyl and fenoxycarb, respectively, against peak areas (*r* values for both calculation curves were higher than 0.9996). Recoveries of the compounds were obtained from two replicate spiking experiments per arthropod group and spiking levels (1 and 100 ng ml^{−1}). Recoveries of fenoxycarb were 120.0 ± 0% for 1 ng ml^{−1} and 92.5 ± 2.1% for 100 ng ml^{−1} in flying insects and 77.5 ± 2.1% for 1 ng ml^{−1} and 88.5 ± 0.7% for 100 ng ml^{−1} in foliage-dwelling arthropods. Recoveries of chlorpyrifos-methyl were 130.0 ± 0% for 1 ng ml^{−1} and 83.0 ± 1.4% for 100 ng ml^{−1} in foliage-dwelling arthropods. Following O'Shea and Johnson [9], the observed recoveries are within the acceptable range for analytical residue analysis. The observed concentrations of the measured residues were not corrected by the observed recoveries. The residues of the nylon bags containing moth scales were added to the residue values of small moths. Residues were normalized to an application rate of 1 kg a.i. ha^{−1} and expressed as residue unit dose (RUD). On the day before the first fenoxycarb application (null measurement), the concentrations of fenoxycarb of all examined arthropod groups were below the quantification limit of 0.002 mg kg^{−1}.

Risk assessment

The risk assessment was performed following the guidance of the European Food Safety Authority (EFSA) on risk assessment for birds and mammals [1].

The exposure, expressed as daily dietary dose (DDD), for acute dietary risk assessment was calculated as the product of the application rate, the peak RUD value resulting from the assumed dietary composition for the species of concern (calculation of the requested 90th percentile was not possible due to the limited data), the food-intake rate per body weight (FIR body wt^{−1}), and a default value of the multiple application factor (MAF) [1]

$$\text{DDD}_{\text{acute}} = \text{application rate} \times \text{RUD (peak value)} \\ \times \text{FIR body wt}^{-1} \times \text{MAF} \quad (1)$$

To assess the reproductive risk, the DDD was calculated in the same way as in Equation 1 but with the difference that the mean peak RUD for the diet of the respective species group and an additional default values for time-weighted average (TWA)

were used. The default value for TWA was 0.53 and assumed a DT50 (time for 50% degradation) of the residue of 10 d [1]:

$$\begin{aligned} & \text{DDDreproductive} = \text{application rate} \\ & \times \text{RUD (mean peak value)} \times \text{FIR body wt}^{-1} \\ & \times \text{MAF} \times \text{TWA} \end{aligned} \quad (2)$$

The MAF value depends on the application interval and the number of applications (MAF for two applications and an application interval of 14 d = 1.2 for acute risk assessment and 1.4 for reproductive risk assessment) [1].

The diet and by that the RUDs differ among the bat species recorded in the examined orchard. In the following, small flying insects are considered to constitute equal shares of small moths and other small flying insects such as flies, beetles, and midges. *Pipistrellus pipistrellus* is known to feed unselectively on available flying insects (with a preference for midges) by aerial hawking [19] but may take some prey items by gleaning [20]. Therefore, we assumed diet compositions consisting mainly of small flying insects and a 5 to 10% fraction of foliage-dwelling arthropods. *Myotis mystacinus* takes swarming small insects (mainly midges) by hawking, but the inclusion of many non-volant prey items indicates also a gleaning habitat [20], while *M. nattereri* is considered to take its prey mainly by gleaning [21]. Hence, the diet of *M. mystacinus* is assumed to consist of 40 to 50% small flying insects, with the remaining 50 to 60% being foliage-dwelling arthropods, and that of *M. nattereri* is assumed to consist of 20 to 30% small flying insects, with the remaining 70 to 80% being foliage-dwelling arthropods. Species of the *Nyctalus-Eptesicus* group are adapted for open-air foraging. Small flying insects such as midges are the main prey for all three species, but moths are also important constituents of the prey of *N. noctula* and *E. serotinus* [20]. To assess the dietary exposure of the group *Nyctalus-Eptesicus*, we considered combinations of small flying insects and large moths, with shares for each prey category between 25 and 75%.

Due to the high energetic cost of aerial foraging, bats require high daily FIRs, estimated as being 70% of their body weight [22]. Pregnancy and lactation are additional energy-demanding processes and require an increase in the FIR of females to up to 85% of the body weight during the lactation phase [23]. Considering that the application took place in the pregnancy and lactation period of bats, we assumed an FIR per body weight of 85%.

The TER values for acute dietary and reproductive risk assessment were calculated as the ratio of toxicity endpoints to the exposure [1].

$$\text{TER}_{\text{acute}} = \text{LD50}/\text{DDD} \quad (3)$$

$$\text{TER}_{\text{rep}} = \text{NOAEC}/\text{DDD} \quad (4)$$

If the TER is larger than the trigger values (10 for acute and 5 for reproductive risk assessment), the risk is considered to be low [1]. The toxicity values for fenoxycarb used in the calcu-

lations were the LD50 (rat, *Rattus norvegicus*; >10,000 mg/kg body wt) and the most sensitive NOAEL determined for fenoxycarb (NOAEL = 5.3 mg/kg body wt/d for long-term study with mice, *Mus musculus*) [24]. No risk-assessment approach was performed for chlorpyrifos-methyl because the measured residues were limited to foliage-dwelling arthropods with the aim of comparing the RUD values of two different pesticides on the same arthropod group.

In the first-tier risk assessment, it is assumed that individuals collect all their food in the treated area (worst-case scenario). In reality, individuals foraging in the agricultural landscape may visit a variety of habitats within a single night and may obtain their food also in a variety of nonagricultural habitats. To consider this, there are possibilities of using more realistic estimates of the proportion of an animal's daily diet obtained in the habitat treated with pesticides in higher-tier risk assessment [1]. Bat activity data obtained by acoustic detection do not allow any conclusions about the amount of time an individual stayed at the examined site. However, following the literature, *P. pipistrellus*, *M. nattereri*, and *M. mystacinus* forage in up to 2.4 [17], 6 [16], and 12 [16] different foraging areas per night, respectively. If we assume that each foraging area is used in the same proportion, and in a best-case scenario, only one sprayed orchard site is used per night, 42, 17, and 8% of the daily food intake of an individual of, respectively, *P. pipistrellus*, *M. nattereri*, and *M. mystacinus* are likely to be contaminated with pesticides. The species of the group *Nyctalus-Eptesicus* are known to feed in extensive foraging areas and to use only very profitable foraging beats such as ponds intensively [16]. We therefore assume that members of that group spend less time in the orchard than all the other species discussed above (i.e., obtain <8% of their food from the treated area).

RESULTS

Bat activity

The most common bat species recorded in the study orchard was the common pipistrelle (*P. pipistrellus*). The serotine (*E. serotinus*), Leisler's bat (*N. leisleri*), and the noctule (*N. noctula*), here compiled as *Nyctalus-Eptesicus*, and the whiskered bat (*M. mystacinus*) and Natterer's bat (*M. nattereri*), which were assigned to the group *Myotis mys-nat*, were recorded frequently (Table 1). All these species were also recorded in the nonagricultural habitats (Table 2). Bechstein's bat (*Myotis bechsteinii*), a species known to forage only within forests, was recorded on few occasions in the forest habitats (data not shown).

In the nonagricultural sites, high activity levels of *P. pipistrellus* were only recorded in the forest-edge habitats (Table 2). Activity levels of *P. pipistrellus* recorded at the orchard sites were on average six times lower than those at the forest edges but approximately 23 times higher than those of the forest and meadow sites (Tables 1 and 2). At two sites (Or1 and Or2) high activity levels comparable to the activity levels of the forest-edge habitats were demonstrated.

Table 1. Recorded mean bat activity (seconds per night) at the eight sampling sites of the study orchard (sites Or1–Or8)^a

Orchard sites	Site Or1	Site Or2	Site Or3	Site Or4	Site Or5	Site Or6	Site Or7	Site Or8	Mean	SD
<i>Pipistrellus pipistrellus</i>	348.1	313.8	41.7	36.2	6.5	7.0	6.9	11.6	96.5	145.6
<i>Myotis mys-nat</i>	7.1	6.5	3.6	4.5	0.8	2.9	2.2	4.2	4.0	2.1
<i>Nyctalus-Eptesicus</i>	9.0	16.3	8.5	9.5	12.5	8.8	5.6	7.0	9.7	3.3

^aMean values are based on eight sampling nights. The resulting mean values for the habitat "orchard" and standard deviations (SDs) are given.

Table 2. Recorded mean bat activity (seconds per night) at the six sampling sites of the nonagricultural habitat sites^{a,b}

			Mean	SD
	Site Fo1	Site Fo2		
<i>Pipistrellus pipistrellus</i>	1.5	7.0	4.3	3.9
<i>Myotis mys-nat</i>	4.8	6.0	5.4	0.9
<i>Nyctalus-Eptesicus</i>	0.2	4.7	2.4	3.2
	Site Ed1	Site Ed2		
<i>Pipistrellus pipistrellus</i>	299.4	923.9	611.7	441.6
<i>Myotis mys-nat</i>	17.4	19.9	18.7	1.7
<i>Nyctalus-Eptesicus</i>	8.0	25.3	16.7	12.2
	Site Me1	Site Me2		
<i>Pipistrellus pipistrellus</i>	6.9	1.5	4.2	3.9
<i>Myotis mys-nat</i>	0.9	0.3	0.6	0.4
<i>Nyctalus-Eptesicus</i>	4.4	35.8	20.1	22.2

^a Mean values are based on three sampling nights. The resulting mean values for the habitats and standard deviations (SDs) are given.

^b Forest sites Fo1, Fo2; forest-edge sites Ed1, Ed2; meadow sites Me1, Me2.

Individuals of the group *Nyctalus-Eptesicus* preferred the meadow and forest-edge sites and avoided the forest (Table 2). At the orchard sites, activity levels of that group were on average between those of the preferred nonagricultural sites, the meadow (two times lower), and the avoided forest sites (four times higher) (Tables 1 and 2).

Individuals of *Myotis mys-nat* showed a strong preference for forest-edge habitats and an avoidance of the meadow sites (Table 2). Individuals recorded at the orchard sites showed also activity levels between those of the preferred nonagricultural sites, the forest-edge sites (four to five times lower), and the avoided meadow sites (six to seven times higher) (Tables 1 and 2).

In the orchard, the highest activity levels of *P. pipistrellus* (1,435.0 s night⁻¹, site Or1) and *Myotis mys-nat* (18.4 s night⁻¹, site Or1) were recorded on the night following the second application of fenoxycarb (day 0) and that of *Nyctalus-Eptesicus*

(33.5 s night⁻¹, site Or5) on the night of day 4 of the first application.

Insecticide residue on arthropods

The RUD values determined for the different arthropod samples are provided in Table 3. The RUDs of all examined arthropod groups reached their peak in the samples taken directly after the application (day 0) with the exception of small moth samples, which revealed higher residue on day 1 postapplication. Foliage-dwelling arthropods exhibited the highest peak values, 20 to 50 times higher than in the other arthropod groups. With time the residues decreased in all of the arthropod groups. The RUDs measured at day 8 decreased to one-tenth for small moths, one-twentieth for other small flying insects, one-sixtieth for foliage-dwelling arthropods, and at least one-one hundred and thirtieth for large moths compared to the respective peak values. The RUDs of large moth samples were below the quantification limit of 0.002 mg kg⁻¹ from day 8 postapplication onward.

The initial value of fenoxycarb for foliage-dwelling arthropods was 13 to 31 times higher than the respective average value for chlorpyrifos-methyl. The initial value of chlorpyrifos-methyl decreased to one-tenth of the initial value within 8 d.

Risk assessment

The calculated TER values of the acute risk-assessment approach of fenoxycarb for the examined combination of species and assumed diet compositions were approximately 61 to 2,254 and 357 to 32,680 times higher than the trigger value of 10 in the worst-case (individuals collect all their food in the treated area) and best-case (individuals collect their food not only in the treated area but in a species-specific number of different foraging areas) scenarios, respectively (Table 4). The TER values of the reproductive risk assessment were below the trigger value of 5 for *M. mystacinus* and *M. nattereri* under the complete range of assumed dietary compositions (Table 4) in the worst-case scenarios. For *P. pipistrellus*, the TER trigger

Table 3. Residue per unit dose (RUD) values (mg kg⁻¹ kg a.i. ha⁻¹) measured from different nocturnal arthropod samples collected from an orchard following two fenoxycarb and one chlorpyrifos-methyl applications^a

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 8	Day 12
Fenoxycarb							
Small flying insects							
1. Application	2.90 ^b	1.04	0.28	0.31	0.29	0.11	0.04
2. Application	2.20	1.75	0.21	0.49	0.37	0.11	0.06
Mean	2.55 ^c	1.40	0.25	0.40	0.33	0.11	0.05
Small moths							
1. Application	No data	4.05	0.91	1.65	0.62	0.64	0.07
2. Application	4.92	7.28 ^b	No data	3.68	2.51	0.37	No data
Mean		5.67 ^c		2.67	1.57	0.51	
Large moths							
1. Application	2.21 ^b	0.89	0.13	0.45	0.14	<0.002 ^d	<0.002 ^d
2. Application	1.34	0.88	0.21	0.12	0.01	<0.002 ^d	<0.002 ^d
Mean	1.77 ^c	0.88	0.17	0.28	0.08	<0.002	<0.002
Foliage-dwelling arthropods							
1. Application	57.52	10.8	11.55	2.71	1.61	1.56	No data
2. Application	133.15 ^b	27.37	8.51	3.47	2.25	1.76	No data
Mean	95.33 ^c	18.72	10.03	3.09	1.93	1.66	
Chlorpyrifos-methyl							
Foliage-dwelling arthropods							
1. Application	4.34	No data	No data	No data	No data	0.15	No data

^a In the performed risk-assessment approach, small flying insects are considered to constitute equal shares of small moths and other small flying insects.

^b Maximum values were used for acute risk assessments.

^c Maximum mean values were used for reproductive risk assessments.

^d Values were below the quantification limit of 0.002 mg/kg.

Table 4. Toxicity exposure ratios (TERs) of fenoxycarb for several bat species and species groups based on their assumed diet compositions^a

Species	Range of assumed diet	Acute risk assessment				Reproductive risk assessment			
		Worst case		Best case		Worst case		Best case	
		RUD	TER _{acute}	RUD	TER _{acute}	RUD	TER _{repro}	RUD	TER _{repro}
<i>Pipistrellus pipistrellus</i>									
	95% flying insects, 5% foliage-arthropods	11.5	5,683.4	4.8	13,616.6	8.7	6.4	3.6	15.6
	90% flying insects, 10% foliage-arthropods	17.9	3,651.4	7.5	8,714.6	13.2	4.2 ^b	5.6	10.0
<i>Myotis mystacinus</i>									
	50% flying insects, 50% foliage-arthropods	69.2	944.5	5.5	11,883.5	49.7	1.1 ^b	4.0	14.0
	40% flying insects, 60% foliage-arthropods	82.0	797.1	6.6	9,903.0	58.8	1.0 ^b	4.7	11.9
<i>Myotis nattereri</i>									
	30% flying insects, 70% foliage-arthropods	94.8	689.4	16.1	4,059.6	67.9	0.8 ^b	11.5	4.9 ^b
	20% flying insects, 80% foliage-arthropods	107.6	607.4	18.3	3,571.6	77.1	0.7 ^b	13.1	4.3 ^b
<i>Nyctalus-Eptesicus</i>									
	25% flying insects, 75% large moths	2.9	22,537.8	0.2	326,797.4	2.4	23.3	0.2	280.1
	75% flying insects, 25% large moths	4.4	14,854.4	0.4	163,398.7	3.5	16.0	0.3	186.7

^aThe combinations of prey groups resulting in the lowest and highest residue per unit dose (RUD) values within the range of assumed species-specific diet compositions are shown. The peak RUD and the mean peak RUD were used for the acute and reproductive risk assessment approaches, respectively. Small flying insects are considered to constitute equal shares of small moths and other small flying insects. In the worst-case scenario it is assumed that the individuals collect all their food in the treated area. In the best-case scenario it is assumed that individuals use a species-specific number of different foraging habitats per night.

^bTER values indicate that they are below the trigger value (10 for acute risk assessment, 5 for reproductive risk assessment).

value of five was reached in the worst-case scenario for dietary compositions which included approximately 7.5% foliage-dwelling arthropods (Table 4). The assumed range of possible diet compositions for the group *Nyctalus-Eptesicus* did not result in a TER value below the trigger value. Under the best-case assumption only the TER value of *M. nattereri* remained below the trigger value (Table 4).

DISCUSSION

Evidence of pesticide exposure to bats

Estimating the risk of pesticides to bats requires linking the occurrence of contaminated food items and the extent of foraging activity. When comparing recorded bat activity levels of the examined orchard to activity levels in nearby habitats known to be used for foraging, we could verify that bats generally used the orchard for foraging during the time period of the fenoxycarb applications. The highest activity levels proven at the orchard for *P. pipistrellus* and *Myotis mystacinus* were recorded in the night (including dusk) following the second application of fenoxycarb, which lasted until dusk. This indicates that bats were not disturbed by the agricultural activity (e.g., tractor application). Considering that most arthropod groups revealed peak residue values on the night following application, avoidance of food items with pesticide residues seems unlikely.

For *P. pipistrellus*, known as a generalist in exploiting foraging habitats [25], we recorded especially high activity at the forest-edge and two orchard sites. Edges of deciduous forest offer flying zones and provide help for acoustic orientation, making them suitable foraging habitats for bats in general [26]. The two orchard sites with remarkably high activity levels (Or1 and Or2, Fig. 1) offered a free flying zone and shelter from all sites. Therefore, this orchard area appeared to exhibit structural features beneficial for foraging comparable to the forest edges.

Both *M. nattereri* and *M. mystacinus* showed a similar use of the orchard and forest sites, while forest edges were strongly preferred and meadow sites avoided. These results are consistent

with the literature, which states that both bat species feed partly by gleaning arthropods from vegetation [20,21]. Furthermore, they are reported to forage along vegetation edges, in orchards and forests [16].

The activity levels measured in the orchard sites for the *Eptesicus-Nyctalus* group, which is adapted to open-air foraging and known to use a wide range of habitats [25], were lower than those in the preferred habitat, the meadows, but higher than those in the forest sites.

Insecticide residue on food items of bats

Foliage-dwelling arthropods exhibited the highest initial residue values. Apart from the exposure during application, it is likely that they experienced additional exposure by crawling on fresh residues on leaf surfaces directly after application.

The surface-to-volume ratio explains the lower initial residue values of the large moths compared to small moths and other small flying insects. Different from other arthropods, the wings of moths (and butterflies) are covered with high numbers of scales. The extensive surface of these scales results in a larger exposure surface and caused higher initial residue values of samples of small moths compared to those of other small flying insects, which were of comparable body sizes. Residue values also depend on the mode of action of the applied pesticide as shown by the differences in the measured initial residues of chlorpyrifos-methyl and fenoxycarb on foliage-dwelling arthropods. Chlorpyrifos-methyl is an acetylcholinesterase inhibitor [27], and most of the arthropods that receive direct exposure die soon thereafter. Thus, following an application, the surviving arthropods that were collected showed low residues. Fenoxycarb, contrarily, is a juvenile hormone mimic in insects, acting as a growth disrupter, and does not kill adult insects but targets juvenile life stages [27]. Therefore, after application of fenoxycarb, up to 31 times higher residue levels were demonstrated in the present study.

The observed decline of arthropod residues over time depends on the persistence of the compound as well as on the dilution of contaminated arthropods with uncontaminated ones. The latter depends on the number of hatching individuals

after the application and the ratio of emigration and immigration of arthropod individuals into the orchard. Our results demonstrated the fastest decline in samples of large moths, a group known to move distances of some 100 m per night [28]. The slowest decline was recorded for samples of small moth species, mainly comprising pest species adapted to reproduce in apple orchards (such as the codling moth) and, therefore, not expected to move out of the orchards. The TWA default value used in the current risk-assessment approach assumes a DT50 of 10 d [1]. Hence, the exposure of bats to fenoxycarb and chlorpyrifos-methyl may be overestimated in the present reproductive risk-assessment approach. However, it is possible that other compounds may be more persistent and exhibit similar initial residue values but slower declines.

In conclusion, the initial value and the residue decline of a particular arthropod group (e.g., flying insects) in a particular crop depends on the arthropod type (e.g., moths or flies in the case of flying insects), their surface-to-volume ratio, their mobility, and the mode of action and persistence of the applied pesticide. Taking this information into account will result in more realistic risk quantification for oral exposure to mammals and birds and especially bats, the only European mammals feeding on vegetation arthropods and flying insects. The guidance document for risk assessment [1] provides only RUD values for ground-dwelling and foliage-dwelling invertebrates but not for any flying insects. Compared to the generic peak RUD value for foliage-dwelling arthropods (mean = $21 \text{ mg kg}^{-1} \text{ kg ha}^{-1}$) [1], the peak values obtained in the present study for the same arthropod group were more than four times higher for fenoxycarb (mean = $95.3 \text{ mg kg}^{-1} \text{ kg ha}^{-1}$) and five times lower for chlorpyrifos-methyl ($4.3 \text{ mg kg}^{-1} \text{ kg ha}^{-1}$). The EFSA values are said to be based on several studies and do not allow any conclusion of the examined arthropod type, their surface-to-volume ratio, the type of pesticide applied (e.g., fungicide or insecticide), the time of application, and the mode of action of the respective pesticide. Those generalized estimates of residue concentration on arthropods introduce uncertainty into the risk assessment.

Risk assessment

Using our feeding guild-specific RUDs, the first-tier approach of the acute dietary risk assessment indicated a low risk for all examined scenarios. However, there is uncertainty if the applied safety factor of 10 used in the TER approach of acute toxicity accounts for interspecific variability in sensitivity [29,30]. Based on LD50 values for two organophosphate insecticides, which were shown to be higher for bats than for laboratory mice, bats are not thought to be more sensitive to pesticides than other mammals in terms of acute dietary toxicology [31,32]. On the other hand, the same authors stated that the surviving bats of those experiments had a more prolonged period of loss of coordination than the laboratory mice. Further research on the acute risk of other pesticide groups to bats would be needed for a more profound conclusion. However, given the high TER values we obtained even under the assumption that individuals were feeding exclusively in the treated field, an acute dietary risk of fenoxycarb appears unlikely.

The TER approach of the reproductive risk assessment indicated unacceptable risk under the worst-case assumption for both *Myotis* species and *P. pipistrellus* but not for the members of the *Nyctalus-Eptesicus* group. All species with a potential risk were assumed to obtain parts of their diet by gleaning foliage-dwelling arthropods, the arthropod group that exhibited the highest residue values by far. The extent of

gleaning is not known for *P. pipistrellus*, but our calculation indicated that shares of approximately 7.5% and more would result in potential risk.

To calculate a refined TER, assumptions were made about the minimal time (best-case scenario) an individual of a particular bat species feeds in the orchard. These assumptions are speculative and radiotelemetry should be carried out to gain insight into bat foraging habits and to enable a more realistic risk-evaluation process. However, our approach helps to place the TER values obtained under assumed best-case scenarios in relation to the trigger value. For *M. nattereri*, the refined TER values were still below the trigger value of 5, while values for *M. mystacinus* and *P. pipistrellus* ranged between 10.0 and 15.6.

The justification of the applied trigger value of 5 for reproductive risk assessment to account for between-species variation in toxicity has also been criticized [33], especially because the information on chronic effects on mammals is to a great extent based on representatives of only one order, the rodents (rat and mouse). Considering that Luttik et al. [33] suggested that interspecies variability for chronic toxicity is at least as variable as that for acute toxicity, for which safety factors up to 15 for mammals were proposed [30], we cannot exclude a reproductive risk even under the assumed best-case scenario for *M. mystacinus* and *P. pipistrellus*.

No endpoints from reproductive toxicity studies of bats are available to allow any deduction on differences in sensitivity compared to other mammals. Moreover, no conclusion can be drawn from LD50 values because it has been demonstrated that the relative sensitivity established from acute tests could be reversed in the case of long-term toxicity as shown for two bird species [33]. However, bats may be especially sensitive to pesticides due to their ecological traits [34]. They differ in many aspects from rodents commonly used in laboratory tests and from shrews used as a surrogate for insectivores requiring high food-intake rates. Most bat species have long life spans and therefore more time for contact with, or accumulation of, dangerous levels of pesticides [31]. Their low reproductive rates (usually a single offspring per year) require high adult survival to avoid population declines [35] and dictate slow recovery of impacted populations. Bats also differ from rodents and other insectivorous mammals such as shrews by physiological constraints due to hibernation and migration. Lipophilic pesticides can have a detrimental effect by accumulating in the stored fat due to the consumption of arthropods contaminated with pesticides. When fat is metabolized during hibernation or migration, pesticide concentrations can reach high and toxic levels, especially in the brain [31]. Moreover, substances that could increase metabolic rates may affect bats that rely on lowered metabolic rates during daily torpor by disrupting energy budgets [9]. These life-history traits can render bat populations more susceptible to long-term effects of pesticides than other mammals.

Additional uncertainties

In the current risk-assessment approach, it is assumed that exposure to pesticides occurs exclusively via diet and not through skin contact or inhalation, although such routes may be relevant under field conditions [36]. Compared to day-active mammals, a higher risk with regard to direct inhalation and dermal exposure may exist for bats as it is common practice to apply pesticides at dusk to avoid, for example, effects on honeybees. Moreover, our results demonstrated that bats were not disturbed by machinery during the application.

Birds and mammals in general may encounter a mixture of different active substances, applied to different crops at different times. This may cause a risk that is not considered to date. Other than the currently used deterministic calculation of toxicity to exposure ratios of one compound, ecological models can integrate factors such as landscape structures, timing of different application, and ecological traits of the organism, and may have the potential to become important tools for the prediction of long-term effects on a landscape scale for birds and mammals in general [37] and bats in particular.

CONCLUSION

For the first time exposure and potential reproductive risk of several feeding guilds of European bats to pesticides were indicated by demonstrating foraging activity and simultaneously measuring residues of two insecticides on the respective food items. Given their ecological traits, bats are potentially more sensitive to reproductive effects of pesticides than other mammals. Therefore, we strongly suggest consideration of bats in the risk-assessment scheme for pesticides and a thorough research program to investigate the effects of different pesticides on the different feeding guilds of bats on a landscape scale.

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Erklärung

Hiermit versichere ich, dass ich die eingereichte Dissertation selbstständig und ohne unerlaubte Hilfsmittel verfasst habe.

Anderer als der von mir angegebenen Hilfsmittel und Schriften habe ich mich nicht bedient.

Die vorliegende Arbeit wurde weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zwecke der Promotion oder eines Prüfungsverfahrens vorgelegt.

Peter Stahlschmidt

Landau, 5.9.2012

Curriculum Vitae

Name: Peter Stahlschmidt



Current address: Jahnstr. 19
76865 Rohrbach
Germany

- 2008–2012 PhD study at the Institute for Environmental Sciences, University Koblenz-Landau about bat activity in agricultural environments and the evaluation of the risk of pesticides impacts.
Biodiversity of noctuid moths and snails in the agricultural landscape (in cooperation with Melanie Hahn and Klaus Swarowsky, respectively).
Participation on marine expeditions to Madagascar (ATIMO VATAE 2010), Saudi Arabia (RED SEA SURVEY 2012) and Papua New Guinea (MADANG 2012). Organisation of a sampling trip to Sulawesi (Indonesia; 2010) to establish a standardized underwater method to sample dead assemblages of shelled molluscs.
- Since 2008 Research Associate at the Muséum national d'Histoire naturelle Paris: Taxonomy and Biogeography of turrids (Gastropoda: Conoidea & Turroidea): revision of the turrids from the PANGLAO 2004 expedition to the Philippines. Correspondence course to graduate in business management (Fern-Universität Hagen).
- 2006 Practical training at RIFCON GmbH (Mammal and bird survey in higher tier wildlife field study in France).
- 2005-2007 Marine Biodiversity Research. Research stays in the Philippines, Mozambique and Brazil.
- 2005 Diploma, Biology. University Kaiserslautern, Kaiserslautern, Germany. (Molecular regulation of the CCM1 gen, a key regulator of the CO₂-Concentration-Mechanism of the green algae *Chlamydomonas*).
- 1998-2005 Student of Biology at the University of Kaiserslautern, Kaiserslautern, Germany.

Publications

Ecology and Ecotoxicology

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