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Dietary analysis of five insectivorous bat species from Kamphaeng Phet, Thailand.

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Abstract. This study provides a baseline for several bat species in Thailand that have never been studied with regard to diets. The diets of five bat species from Northern Thailand were analysed based on faecal pellets. The percentage volume of insect orders in the diet of *Hipposideros armiger* (30 pellets) consisted for 28.2% of Hemiptera, 20.5% of Lepidoptera and 20.1% of Coleoptera. The diet of this species was not dominated by a single order. The diet of *Hypsugo cadornae* (30 pellets) consisted for 49.3% of Hemiptera and 43.7% of Coleoptera. *Rhinolophus thailandensis* (31 pellets) had remains in its pellets consisting of 43.2% Coleoptera, 32.0% Hemiptera and 12.3% Homoptera. *Rhinolophus thailandensis* and *H. cadornae* both feed on large numbers of insects that have thick exoskeletons. For *Taphozous melanopogon* (30 pellets) the diets was comprised of 45.1% Hemiptera, 19.0% Lepidoptera and 15.3% Coleoptera. The diet of *T. longimanus* (30 pellets) consisted of 30.7% Diptera, 23.7% Lepidoptera and 19.0% Hemiptera. The large amount of Diptera and relative large numbers of Culicidae in the diet of this urban population might have potential implications for the control of vector mosquitoes.

Key words. *Hipposideros armiger*, *Hypsugo cadornae*, *Rhinolophus thailandensis*, *Taphozous longimanus*, *Taphozous melanopogon*, diet

INTRODUCTION

Insectivorous bats provide important ecological services such as pest control (Boyles et al., 2011). In the United States, for example, the economic importance of bats in agriculture has been estimated to be as high as US$ 22.9 billion annually (Boyles et al., 2011). The value of bats in agriculture is also likely to be high elsewhere, where agriculture is of importance. For example in Thailand, Leelapaibul et al. (2005) estimated that the total Thai *Tadarida plicata* population feeds on approximately 54.8 tons of insects nightly. The diet of this species consisted of at least 41% of agricultural pest species therefore its impact on pest control can be considered to be significant (Leelapaibul et al., 2005). Some studies have shown that some bat species feed on mosquitoes, which makes them interesting for vector control as well (Rydell et al., 2002; Reiskind & Wund, 2009). Reiskind & Wund (2009) experimented with with caged bats and ovipositing mosquitoes, they found a 32% reduction of egg rafts in areas with bats compared to bat-free areas.

Despite the rich diversity of 104 insectivorous bat species in Thailand (Bumrungsri et al., 2006), little is known about the diets of most of these species. To date, the diet of only a few species have been studied in Thailand, including *Tadarida plicata* (Leelapaibul et al., 2005) and *Taphozous longimanus* (Boonkird et al., 2004). It is therefore of particular interest to acquire information about the types of insects these bats prey upon, because this can have major implications for the biological control of agricultural pest and disease vectors such as mosquitoes.

In the investigation of bat diets, studies show that some species can be considered specialist feeders while most species are generalists (Freeman, 1979; Bogdanowicz et al., 1999; Aguirre et al., 2003; Weterings & Umponstira, 2014). It is thought that certain species prefer soft-bodied insects or moths while others do not show any preference for specific prey items (Freeman, 1979; Bogdanowicz et al., 1999; Aguirre et al., 2003; Ghazali & Dzeverin, 2013; Weterings & Umponstira, 2014). Insectivorous bats can be classified into four categories based on their diet: generalists, hard bodied insect feeders, soft bodied insect feeders and Lepidoptera specialists (Freeman, 1979; Bogdanowicz et al., 1999; Aguirre et al., 2003). However, these categories are not very strict, and diet can be dependent on locality, season and climate (Leelapaibul et al., 2005; Zhang et al., 2005).

The aim of this study was to provide insights into the feeding habits of five bat species occurring in north-western Thailand. We investigated the diet of the great roundleaf bat (*Hipposideros armiger;* Hodgson, 1835), the Cadorna's pipistrelle (*Hypsugo cadornae;* Thomas, 1916), the Thailand

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horseshoe bat (*Rhinolophus thailandensis;* Wu et al., 2009), the long-winged tomb bat (*Taphozous longimanus*; Hardwicke, 1825) and the black-bearded tomb bat (*Taphozous melanopogon*; Temminck, 1841). While the diet of both tomb bat species has been investigated in China, India and Thailand (Boonkird et al., 2004; Srinivasulu & Srinivasulu, 2005; Wei et al., 2008), there are no known published studies on the diet of the other three species investigated in this study.

METHODS

Bat faecal pellets were collected at four different sites in the Kamphaeng Phet province in north-western Thailand. When collecting faeces we searched for the freshest pellets, which were recognised by the darker colour and softer texture. Faecal pellets from *H. armiger* and *R. thailandensis* were collected at two separate areas in a cave in Khong Lan national park on 6 February 2013. This cave consisted of several chambers that were connected via narrow tunnels. Each species occupied different chambers. *Hipposideros armiger* was found in a chamber of which the ceiling reached a height of approximately five to six meters. *Rhinolophus thailandensis* was only found in a chamber of which the ceiling was approximately two meters high. We collected only fresh faecal pellets directly under the roosting bats in order to reduce any chances of cross-species contamination. Faecal pellets from *H. cadornae* were collected from under a sugar palm (*Borassus flabellifer*) on 13 February 2013. This palm was used as roosting site and was located in a rice paddy near the village Lan Dok Mai. Kamphaeng Phet city hosts a large colony of *T. longimanus*, which roosts under the bridge that crosses the Ping River. Faecal pellets from this species were collected under the bridge on 25 January 2013. The faecal pellets from *T. melanopogon* were collected in a very small cave in a predominantly agricultural landscape near Phran Kratai town on 28 January 2013. During several surveys we only found this cave to be occupied by this species, therefore cross-species contamination is unlikely. In addition, we only collected fresh faecal pellets to reduce any chances of cross-species contamination.

Faecal pellets were separately stored in glass containers filled with alcohol. Bats were identified with combined techniques. Echolocation calls were recorded and photographs of the bats were taken. A Petterrson 240X and a Baton (Batbox) Bat Detector with recording devices were used to record the echolocation calls. With help of existing species-presence-absence models (Hughes et al., 2010) we could narrow down the number of species that are likely to be present in the study area. Some species have a strong overlap in their call frequencies, therefore narrowing the number of species was essential in order to reliably identify the species. We used Audacity software (version 2.0.0, Oetzmann & Mazzoni, 2013) for analysing the recorded echolocation calls. The calls were compared with calls from previously identified bats in the region based on published and unpublished data (Furey et al., 2009; Zhang et al., 2009; Hughes et al., 2010; Hughes et al., 2011, Boonman unpublished data). Photographs were then used to corroborate species identification. These combined methods produce reliable results without invasive techniques such as mist netting (Hughes et al., 2010).

Insectivorous bats digest their food very rapidly and do not thoroughly chew their prey. Therefore, faecal pellets often contain many parts of prey items that can still be used to identify the prey to the taxonomic order and sometimes to higher taxonomic levels (Whitaker et al., 2009; Whitaker & Castor, 2010). Identification of prey items from faecal pellets is therefore almost as reliable as stomach content analysis (Whitaker et al., 2009). The advantage of analysing faecal pellets is that this technique is not invasive. We used the guidelines and identification keys provided in by Whitaker et al., (2009) and Whitaker & Castor (2010) for analysing the faecal pellets. We added other insect orders to these guidelines that were common in the area but were not present in the guidelines. Pellets were dissected with a needle and insect parts were identified with help of a digital microscope (CMOS USB Digital Microscope). We identified insect parts to the highest possible taxonomic level, which was often the order but sometimes the family level. We calculated the percentage volume of every insect order or family found in the pellets for each bat species. The percentage frequency per insect order or family was also calculated for each bat species. The percentage frequency represents the percentage of pellets in which a specific insect order or family was found (Whitaker & Castor, 2010). We calculated the ratio of percentage volume versus the percentage frequency to find any discrepancies between the two measures. These ratios were then compared using ANOVA and Tukey’s post-hoc test. All analysis were conducted in Rstudio version 0.98.1028 (RStudio, 2012) built on R 3.0.2 (R Development Core Team, 2013).

RESULTS

A total of 151 faecal pellets were analysed; 30 pellets for *T. longimanus*, *T. melanopogon*, *H. cadornae* and *H. armiger* and 31 pellets for *R. thailandensis*. Insect fragments were identified from 11 insect orders (Table 1). Hemiptera and Coleoptera were the most common food items, occurring in 56.3% and 46.4% of the total number of pellets respectively. Other important insect orders were Lepidoptera (41.7%), Diptera (25.2%) and Homoptera (17.9%).

In terms of percentage volume, the diet of *H. cadornae* consisted mainly of Hemiptera (49.3%) and Coleoptera (43.7%). This was the only species for which we did not find any moth (Lepidoptera) fragments or scales in the faecal pellets. In one faecal sample of this species we found nine individual mites (Acarina), mites were not detected in the pellets of any other species. The percentage volume of Coleoptera in its diet was higher than that of all other species except for *R. thailandensis*. The diet of *T. longimanus* consisted mainly of Diptera (30.7%), Lepidoptera (23.7%), Hemiptera (19.0%) and Coleoptera (14.5%). The amount of Diptera (including Culicidae) in the diet of this species was higher than that of the other species. Diptera occurred in 36.7% of the samples. *Taphozous longimanus* fed on

Table 1: Percentage frequency and volume of all insect orders in bat faeces from the studied species.

Order	Family	*Hipposideros armiger*		*Hypsugo cadornae*		*Rhinolophus thailandensis*		*Taphozous longimanus*		Taphozous melanopogon	
		Volume (%)	Frequency (%)	Volume (%)	Frequency (%)	Volume (%)	Frequency (%)	Volume (%)	Frequency (%)	Volume (%)	Frequency (%)
Acarina		–	–	0.2	3.3	–	–	–	–	–	–
Coleoptera		20.1	34.5	43.7	70.0	43.2	58.1	14.5	30.0	15.3	38.7
Diptera	Unidentified	4.0	13.8	1.2	6.7	5.5	16.1	25.7	46.7	7.4	32.3
	Culicidae	–	–	0.3	3.3	–	–	2.7	3.3	–	–
	Psychodidae	–	–	–	–	–	–	1.3	3.3	–	–
	Tipulidae	–	–	–	–	–	–	1.0	3.3	–	–
	Total Diptera	4.0	13.8	1.2	6.7	5.5	16.1	30.7	56.7	7.4	32.3
Ephemeroptera		4.5	20.7	–	–	1.9	6.5	2.2	6.7	1.9	22.6
Hemiptera		28.2	48.3	49.3	76.7	32.0	45.2	19.0	33.3	45.1	77.4
Homoptera	Unidentified	7.0	24.1	2.5	3.3	2.6	3.2	3.0	10.0	7.1	22.6
	Cicadellidae	8.6	10.3	–	–	9.7	9.7	–	–	3.2	6.5
	Total Homoptera	15.6	34.5	2.5	3.3	12.3	12.9	3.0	10.0	10.3	29.0
Hymenoptera		–	–	2.7	3.3	0.3	3.2	–	–	0.8	3.2
Isoptera		1.6	3.4	–	–	–	–	–	–	–	–
Lepidoptera		20.4	55.2	0.2	3.3	3.5	25.8	23.7	63.3	19.0	61.3
Neuroptera		2.1	3.4	–	–	–	–	–	–	–	–
Orthoptera		–	–	–	–	0.6	6.5	–	–	–	–
Trichoptera		–	–	–	–	0.5	3.2	–	–	–	–
Unidentified		3.6	6.9	–	–	0.2	3.2	7.0	10.0	0.2	3.2

little Hemiptera compared to all other species except *H. armiger*. For *T. melanopogon* the percentage frequency of Diptera (32.2%) was similar to that of *T. longimanus* but the percentage volume (7.4%) was much lower. The diet of *T. melanopogon* mainly consisted of Hemiptera (45.1%) followed by Lepidoptera (19.0%), Coleoptera (15.3%) and Homoptera (7.1%). For *R. thailandensis* the diet mainly consisted of Coleoptera (43.2%), Hemiptera (32.0%) and Homoptera (12.3%) in terms of percentage volume. The diet of this species was the most diverse in terms of insect orders, containing insects from nine different orders compared to eight for *H. armiger*, seven for *H. cadornae*, seven for *T. melanopogon* and five for *T. longimanus*. *Rhinolophus thailandensis* was the only species for which we found insect fragments in the orders Trichoptera (1 pellet) and Orthoptera (2 pellets). The percentage volume of insect orders in the diet of *H. armiger* was highest for Hemiptera (28.2%), Lepidoptera (20.5%) and Coleoptera (20.1%). *Hipposideros armiger* was the only species for which we found Neuroptera (1 pellet) and Isoptera (1 pellet) fragments in the faecal pellets. The percentage volume of Hemiptera was highest for this species.

In the five main food items there were significant differences in the ratio of percentage volume and percentage frequency ($F = 4.84$, $d.f. = 4$, P-value = 0.007): Coleoptera 0.57 ±0.13, Diptera 0.31 ±0.15, Hemiptera 0.62 ±0.06, Homoptera 0.56 ±0.28 and Lepidoptera 0.25 ±0.15. The post-hoc test showed that the ratio of Lepidoptera was significantly lower than that of Coleoptera and Hemiptera. In addition, the ratio for Diptera was also significant lower than that of Hemiptera.

DISCUSSION

Our analyses give some important baseline information about five bat species from Thailand. We found large differences in the diets of some species (e.g., *H. cadornae* vs. *T. melanopogon*) but small difference in the diets of other species (e.g., *H. armiger* vs. *T. melanopogon*). The diets are discussed per species in further detail in the following paragraph. Note that these results should be interpreted carefully as diets of bats are very dependent on region and season (Leelapaibul et al., 2005; Zhang et al., 2005). There is often much variation found in the diet of bats from the same species coming from different regions (Kurta & Whitaker, 1998; Srinivasulu & Srinivasulu, 2005). It is therefore difficult to use such information to make generalisations about one species.

When comparing the percentage volume and frequency of occurrence of prey items for this study, the ratio appeared to be lower for Lepidoptera and Diptera in comparison to Coleoptera, Hemiptera and Homoptera. This difference in Diptera can be explained by the fact that Diptera are often smaller and softer than insects from the other orders (Lease & Wolf, 2010). A single dipteran prey will take up less volume in a faecal pellet but will still be counted as present resulting in a higher volume-frequency ratio. For Lepidoptera, this difference could be explained by the fact that the wing scales often remain in the digestive tract for a longer period (Whitaker et al., 2009). This results in a low Lepidoptera percentage volume because only a few scale are found, but in a higher frequency because they are present in many faecal pellets. These differences can cause a bias when interpreting the diet of bats. It is therefore important to consider both percentage volume and percentage frequency because both measures capture different and additive information.

***Taphozous longimanus* and *Taphozous melanopogon*.** We found high numbers of Diptera in the diet of *T. longimanus* (30.7% volume), which was contrary to previous studies that found that bats that feed on large numbers of Diptera and Lepidoptera are often small and have high call frequencies (Bogdanowicz et al., 1999; Weterings & Umponstira, 2014). In constrast, *T. longimanus* is a relative large insectivorous bat, with a mean body weight of 27 g (Smith et al., 2003), and has a relatively low call frequency (frequency of maximum energy is 30.8 kHz; Hughes et al., 2011). Large bats with low call frequencies are more likely to have a diet that consists mainly of insects with thick exoskeletons such as Coleoptera or Hemiptera. The similarly sized *T. melanopogon* also had relatively high Diptera content in the diet (7.4% volume), although it was not as high as for *T. longimanus*.

Diet studies of the two species seem to indicate that Diptera content may vary owing to habitat or seasonal availability. Srinivasulu et al (2005) investigated the diet of *T. melanopogon* in two different habitat types and found higher Diptera content for diets from bats roosting in a semi-urban habitat compared to those in a forest habitat. A Chinese study also investigated the diets of *T. melanopogon* and found that Diptera also made up a relative large part of the diet of this species, with an overall percentage frequency of 11.1% during a five-month period that had a single month peak of 73.2% (Wei et al., 2008). On the other hand, in Bangkok the diet of *T. longimanus* was found to only consist of 2.54% Diptera and 53.04% of Lepidoptera (Boonkird et al., 2004). Hence, it is likely that both *T. longimanus* and *T. melanopogon* may be opportunistic feeders that can exploit a wide diversity of available insects.

The high Diptera content in the diet of *T. longimanus* is interesting especially because the sampled population is located in an urban area. Culicidae made up at least 8.8% of the total volume of Diptera found in this species therefore it might be potentially important in the control of urban disease vectors in this insect family.

***Hypsugo cadornae*.** The diet of *H. cadornae* mainly consisted of Coleoptera and Hemiptera but did not contain any Lepidoptera. This diet is in line with what would be expected based on the the relative low call frequency (37.5 kHz, Boonman unpublished data), which is an indication towards low Lepidoptera content and a high content of insects with thick exoskeletons (Bogdanowicz et al., 1999; Weterings & Umponstira, 2014). The species is relative small with a mean body mass of 5.9 g and a forearm length of 12.9 mm (Francis, 2008). Small bats are more agile and therefore more likely to feed on smaller insects, this might for example explain some of the Culicidae content in its diet.

The presence of mites (Acarina) in one faecal pellet was also very interesting. Mites are common parasites on bats and have reported for a range of bat species (Whitaker et al., 1983). There are many species of mites that are associated with bats (Baker & Craven, 2003). Not many studies have reported the presence of mites in faecal pellets, nevertheless, they are commonly found on bats and could possibly end up in the faeces if ingested during grooming (Miková et al., 2013). For some bat species it is know that mite infestations increase grooming activities (Giorgi et al., 2001; Godinho et al., 2013). Mites are relative small and will mostly remain intact while passing the digestive tract of a bat. Therefore, they would easily be recognised when they are present in faecal pellets and it would be very likely that they would have been reported more often. Hence, the fact that mites were only found in a single faecal pellet together with the high number of mites (nine) in one pellet suggests that these mites are likely not parasites from the bat but from one of the food items. The faecal pellet that contained these mites consisted for 95% of Coleoptera fragments. Parasitic mites are also found on Coleoptera (Abbot & Dill, 2001; Almane & Elnov, 2009). In addition, we did not find hairs in the faecel pellet which would have been likely in case the mites would have ended up in the diet due to grooming (Mikova et al., 2013). Therefore, it can be assumed that these mites may have been present on one or more food items.

***Rhinolophus thailandensis* and *Hipposideros armiger*.** The dietary analysis of *R. thailandensis* has not been conducted previous to this study. Apart from several taxonomic publications and occurrence records little is known about this species. The species shows a very close resemblance to *R. pearsoni* and *R. yunanensis* and is often confused (Zhang et al., 2009). One Chinese study that focused on the diet of *R. pearsoni* found that the main components of the diet of this species were Lepidoptera (48%) and Coleoptera (34%) (Zhou et al., 2002). *Rhinolophus thailandensis* in our study only had a small amount of Lepidoptera in its diet. More interesting are the differences between this species and *H. armiger*, as the faecal samples were collected approximately 50 m away from each other. *Hipposideros armiger* fed on significantly more Lepidoptera and less Coleoptera in comparison with *R. thailandensis*. It could be possible that both species could have different feeding grounds which might result in differences in diets (Burland et al., 2006). Another possibility is that one or both species are selectively choosing their prey.

Our study gives some insights in the diets of five bats species from Thailand. The diets of some of these species have never been studied before, and this study provides useful baseline information about these species. We observed large amounts of Diptera content in the diet of an urban population of *T. longimanus*, which included a considerable amount of mosquitoes (Culicidae). It is possible that urban bat populations could play a role in the control of certain vector mosquitoes. Elucidating the role of bats in mosquito vector control could possibly lead to benefits for both the conservation of bats and public health.

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