Niche of Sympatric Insectivorous Bats in Central Thailand Revealed by

Carbon and Nitrogen Stable Isotopes

Nittaya Ruadreo

A Thesis Submitted in Partial Fulfillment of the Requirements for the

Degree of Master of Science in Ecology (International Program)

Prince of Songkla University

2018

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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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ชื่อวิทยานิพนธ์ ชีพพิสัยของค้างคาวกินแมลงที่อยู่ร่วมกันในภาคกลางของประเทศไทย โดยการใช้
ไอโซโทปเสถียรคาร์บอนและไนโตรเจน
ผู้เขียน นางสาวนิตยา รวดเร็ว
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<div align="center">**บทคัดย่อ**</div>

การกินอาหารเป็นสิ่งจำเป็นต่อการอยู่รอดของสัตว์ สัตว์ที่มีการอาศัยอยู่ร่วมกันจึงมีวิธีการที่หลากหลายเพื่อใช้ในการแบ่งปันชีพพิสัย (ขอบเขตของสิ่งมีชีวิตที่ทนได้ต่อกลุ่มของปัจจัยสิ่งแวดล้อมที่เป็นปัจจัยจำกัด การเข้าถึงอาหารและพลังงาน รวมทั้งพฤติกรรมที่แสดงออกมาตอบสนองต่อสิ่งมีชีวิตอื่นๆ) ทั้งในเชิงพื้นที่และเวลา การศึกษาไอโซโทปเสถียรสามารถบ่งบอกการแบ่งปันชีพพิสัยในพื้นที่หากินและลำดับการบริโภคของค้างคาวกินแมลงที่ใช้พื้นที่หากินเดียวกันได้ การศึกษาในครั้งนี้ชี้ในเห็นชีพพิสัยของค้างคาวที่อยู่ในพื้นที่เดียวกัน ซึ่งได้แก่ ค้างคาวหน้ายักษ์สามหลีบ ค้างคาวปากย่น ค้างคาวปีกถุงเคราดำ และค้างคาวปีกถุงใหญ่ ในภาคกลาง ประเทศไทย โดยการใช้ไอโซโทปเสถียรคาร์บอนและไนโตรเจนจากการวิเคราะห์เนื้อเยื่อปีกและขนของค้างคาว ผลการศึกษาพบว่าเนื้อเยื่อปีกของค้างคาวแต่ละชนิดมีค่าไอโซโทปเสถียรแตกต่างกัน มีผลมาจากความแปรผันของอาหารหลักของค้างคาวแต่ละชนิด ความแปรผันเชิงเวลาในเนื้อเยื่อปีกมีผลมาจากค้างคาวดังกล่าวนี้มีความแปรผันของอาหารในแต่ละฤดูกาล ในฤดูฝนค้างคาวกินแมลงที่อยู่ในพื้นที่เดียวกันกินแมลงที่กินพืช C_4 มากกว่า C_3 อีกทั้งไอโซโทปเสถียรไนโตรเจนมีค่าต่ำกว่าในฤดูร้อน ค่าความกว้างของชีพพิสัยจากขนค้างคาวชี้ให้เห็นว่าค้างคาวหน้ายักษ์สามหลีบมีชีพพิสัยกว้างที่สุด รองลงมาเป็นค้างคาวปีกถุงใหญ่ ค้างคาวปากย่น และค้างคาวปีกถุงเคราดำ ตามลำดับ ในขณะที่เนื้อเยื่อปีกพบว่า ค้างคาวหน้ายักษ์สามหลีบยังคงมีชีพพิสัยกว้างที่สุด รองลงมาเป็น ค้างคาวปีกถุงเคราดำ ค้างคาวปีกถุงใหญ่ และค้างคาวปากย่น ตามลำดับ ชีพพิสัยมีการซ้อนทับกันเพียงเล็กน้อยในค้างคาวทั้งสี่ชนิดแต่มีการซ้อนทับกันมากขึ้นในฤดูร้อน โดยเฉพาะอย่างยิ่งในกลุ่มค้างคาวที่กินแมลงในที่โล่ง (Aerial insectivores) ผลจากเนื้อเยื่อปีกชี้ให้เห็นว่าอาหารหลักที่ค้างคาวหน้ายักษ์สามหลีบกินเป็นหลัก คือ กลุ่มแมลงปีกแข็ง (coleopteran) ในขณะที่ค้างคาวปีกถุงเคราดำ ค้างคาวปีกถึงใหญ่ และค้างคาวปากย่นกินแมลงในกลุ่มยุง (dipteran) เป็นหลัก นอกจากนี้การศึกษานี้ยังเป็นการรายงานนิเวศวิทยาการกินของค้างคาวปีกถุงใหญ่เป็นครั้งแรกอีกด้วย เนื่องด้วยลักษณะทางนิเวศสัณฐานและระบบคลื่นเสียงของค้างคาวนั้นจึงนำไปสู่ความแตกต่างของพื้นที่หากินและเหยื่อของค้างคาวที่ใช้พื้นที่หากินเดียวกันเหล่านี้

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Author   Miss Nittaya Ruadreo

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Abstract

Foraging is critical for animal survival. Sympatric animals adopt various ways for trophic niche partitioning both in space and time. The study of stable isotope signatures can reveal foraging habitat partitioning and trophic structures used by sympatric insectivorous bat species. This study determined niche partitioning of sympatric insectivorous bats namely *Hipposideros larvatus, Chaerephon plicatus, Taphozous melanopogon* and *T. theobaldi* in central Thailand, using carbon and nitrogen stable isotopes from wing tissue and fur. The results showed that wing tissues of each species have different values of isotopic ratio imply interspecific variation in their major diet. Temporal variations in wing tissue infer that these bats altered their diet in different seasons. In wet season, bats foraged more on C_4-feeder insects than C_3-feeder insects and $\delta^{15}N$ ranges were lower in all species compared to dry season. Niche width from fur showed that *H. larvatus* was the greatest followed by *T. theobaldi, C. plicatus* and *T. melanopogon,* respectively while wing tissue indicated that *H. larvatus* was also the greatest following by *T. melanopogon, C. plicatus* and *T. theobaldi,* respectively. Niche was slightly overlapped in these four species but more overlapped in dry season, especially those aerial insectivores. Based on wing tissue, the main diet of *H. larvatus* was coleopteran while *T. melanopogon, T. theobaldi* and *C. plicatus* were dipteran. Moreover, this study provided the first report on feeding ecology of *T. theobaldi*. Their eco-morphological characteristics and echolocation lead to differentiation of habitats and preys of these sympatric bats.

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<div align="right">Nittaya Ruadreo</div>

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

For all animals, feeding is essential for survival and reproduction. The choice of suitable foraging habitats depends on the availability of local food as well as on vegetation structures (Kusch *et al*., 2004). Chiroptera consume a large variety of food items including arthropods such as insects, scorpions and spiders, small vertebrates, fruits, nectar and pollen, and even blood. Accordingly, bats influence terrestrial ecosystems and the structure of arthropod assemblages (Altringham, 2011, Kusch *et al*., 2004). Insectivorous bats prey on nocturnal insects and potentially act as biological control agent on farmland (Leelapaibul *et al*., 2005). Many insectivorous bats are sympatric. Sympatric insectivorous bat species may differ in eco-morphological characteristic, habitat use and prey preferences (Jiang *et al*., 2008). The most common mechanism by which animals divide resources and partition the flow of key elements within food webs is trophic niche partitioning (Schoener, 1986) and dietary niche may differ among and within population and individuals (Nadjafzadeh *et al*., 2015). It is widely assumed that bats partition available microhabitat and food resources based on their wing morphology and echolocation characteristics. Indeed, bats can be separate into functional groups: fast-flying hawking, slow-flying hawking, trawling, gleaning and hovering, fly-catching and perch hunting bats. The evolution of echolocation call design is also strongly influenced by flight, food and foraging habits. The differences in wing morphology and echolocation are largely a reflection of foraging strategy of the bat– where they feed, how they feed and what they feed on (Altringham, 2011).

Understanding the food preferences of insectivorous bats has largely been limited to analysis of stomach and fecal contents (Whitaker, 1995). There have been several studies of the feeding habits of bats which relied on analysis of insect remains obtained from the stomach contents or from direct observation beneath roosts used by bats. Because of the aforementioned thorough mastication of insects during ingestion, the specific identities of the species eaten were rarely known, although details of the predator, especially for stomach contents, were available (Fenton, 1974). However, this method is difficult and

results cover only a short retrospective time period and for fecal and stomach analysis provides little and no information on habitats used while foraging (Brigham, 1991). To obtain precise information concerning the partitioning of food resources, it is essential to identify accurately what kinds of foods have been consumed.

Stable isotope has been increasingly used to study animal diets over short and long time scale. Variability in $\delta^{15}N$ and $\delta^{13}C$ values in animal originate from differences in the isotopic ratios of carbon and nitrogen in the food web, and through variable expression of isotopic discrimination during the uptake and processing of diet-derived stable isotopes in consumer tissues, which varies according to the metabolic rate of those tissues (Tieszen *et al.*, 1983, Post, 2002). Using stable isotopes, it is possible to assess the trophic level and food web in ecological studies and further to evaluate temporal and spatial variation in diet, food web, and behavior both within and between species (Sullivan *et al.*, 2006). Lastly, analyses of stable isotope ratios in animal tissues can disclose an habitat and resource use during tissue growth (Cryan *et al.*, 2012) and may also integrate dietary information over different time periods according to the specific tissue under investigation, e.g. weeks, months, years in blood, muscle tissue or bone material respectively (Hobson and Clark, 1992). Stable nitrogen ($^{15}N/^{14}N$ or $\delta^{15}N$) and carbon ($^{13}C/^{12}C$ or $\delta^{13}C$) isotope ratios of a consumer tissue indicate trophic level and foraging location. This technique can be used to trace wildlife diets, to analyze food web membership (DeNiro and Epstein, 1981, Popa-Lisseanu *et al.*, 2014) and to evaluate the structure and dynamics of animal communities (Peterson and Fry, 1987). In mammals such as bats, stable isotope ratios can be used to investigate an inert tissue (fur) to provide information about the diet, food web membership and local water sources at the time of molt (Peterson and Fry, 1987).

Several studies about feeding ecology in bats have been done in temperate zone, but none of the study has been conducted in paleotropical insectivorous bats. The focal species of this study, namely *Hipposideros larvatus, Chaerephon plicatus, Taphozous melanopogon* and *T. theobaldi* differ in wing morphology and echolocation call design and these differences might cause them to forage in different habitats. Interestingly, these species roost together in the same caves and may use the same area as a foraging habitat. Here, I used carbon and nitrogen stable isotope analysis to reveal niche partitioning of those sympatric insectivorous bats. Moreover, this study will provide information about the feeding ecology of these species. This basic knowledge about the foraging behavior and

habitat use may be of prime importance to develop conservation practices for these species (Stebbings, 1988).

1.2 Literature reviews

1.2.1 study species

Horsefield's leaf-nosed bat (*Hipposideros larvatus*) is a species in the family Hipposideridae. This species is found at elevations ranging from 182 to 860 m above sea level. It roosts in caves and in abandoned mines, rock crevices, mines shafts, pagodas, buildings, and tropical moist forest. Roosts are often shared with other bat species and may count up to several hundred bats (Bates *et al.*, 2008). Most of the hipposiderid bats have wing shapes that are suitable for foraging in cluttered environments. *Hipposideros larvatus* has broad wings, with the studied population has average body mass 21.5 ± 3.6 g and average forearm length of 62.1 ± 2.0 mm (n = 62, N. Ruadreo, unpublished data).

Black-bearded tomb bat (*Taphozous melanopogon*) is a species of the family Emballonuridae. This species prefers hilly and forested areas, with freshwater and roosts in caves. (Csorba *et al.*, 2008). It is roosting in groups of thousand individuals. This species has narrow wings with average body mass 28.6 ± 3.0 g and forearm length 65.3 ± 1.3 mm (n = 44, N. Ruadreo, unpublished data).

Theobald's tomb bat (*Taphozous theobaldi*) is a species of the family Emballonuridae. In Thailand, it is the largest representative of the genus (Lekagul and McNeely, 1977). This species usually roosts in caves (Bates *et al.*, 2008). Their average body mass is 37.9 ± 3.6 g and their forearm length is 73.2 ± 1.8 mm (n = 40, N. Ruadreo, unpublished data). Similar to other *Taphozous*, this species has narrow wings and was reported to forage at high altitude up to 800 meters (Roeleke *et al.,* 2017).

The wrinkle-lipped free-tailed bat (*Chaerephon plicatus*) is a species within the family Molossidae. Mollosid bat usually forages at high altitudes, i.e. up to several kilometers, and as far as 25 kilometers from its roost (William *et al.*, 1973). This species forms large colonies of thousands of bats in caves. Its body mass is 15.6 ± 1.8 g and forearm length is 47.1 ± 1.1 mm (n = 42, N. Ruadreo, unpublished data). Their narrow wings with wing loading in male of 0.21 - 0.29 and in female of 0.20 - 0.29 g/cm^2 (Leelapaibul *et. al.*,

2003) makes them fast-flying species and hunting in open space such as forested area and over rice fields.

1.2.2 Insectivorous bat and feeding strategies

Bats (order Chiroptera) are more ecologically diverse than any other group of mammals except Rodentia. They have numerous morphological, physiological, and behavioral adaptations of sensory and motor systems which permit bats to access a wide range of habitats and resources at night (Schnitzler and Kalko, 2001). Insectivorous bats form a diverse group, comprising more than 780 species worldwide. Most of them are skilled nocturnal hunters of insects (Neuweiler, 2000) with unique biological characteristics. Southeast Asia is home to over 25% of the world's bat diversity (Kingston, 2013). These species use a variety of habitats both for feeding and roosting. Local bat assemblages include large numbers of species with a high diversity in their foraging area, owing to a large variety in foraging modes and diets. Insectivorous bats are major predators feeding on nocturnal insects and have the potential role as biological pest control agents in farmlands (Leelapaibul *et al.*, 2005). By doing so they may influence the structure of arthropod communities and thus whole terrestrial ecosystems (Altringham, 2011).

The wing shape and echolocation call design is adapted towards the prime insect ressouces a bat species is using (Denzinger and Schnitzler, 2013). Insectivorous bats have been suggested as the primary consumer of nocturnal insects (Kunz and Pierson, 1994). The most common prey insect groups of bats include Lepidoptera, Coleoptera, Homoptera, Hemiptera, and Trichoptera (Kunz *et al.*, 1995). On farmland, prey insects of bats include corn borers, planthoppers, tobacco budworms, and oriental armyworms (Whitaker, 1993). Studies of dietary habits of insectivorous bats date back many years, although there is considerable variation in the relative proportions consumed by different species, some species also eat unusual prey items such as scorpions and spiders. (Kunz *et al.*, 2011). *Tadarida plicata* fed on at least nine insect orders consisting Homoptera, Lepidoptera, Hemiptera, Coleoptera, Diptera, Hymenoptera, Odonata, Orthoptera and Psocoptera, respectively (Leelapaibul *et al.*, 2005). Thus, *T. plicata* potentially may be able to regulate insect populations over a large area (Leelapaibul *et al.*, 2005). For the congeneric bat *T. brasiliensis* it has been shown that they forage at high height above ground (Williams *et al.*, 1973), and at large distances of about 25 km from their roosting caves. *Taphozous*

longimanus has been known as a solitary forager, flying commonly at heights of 25 to 62 m with occasional fast, swooping runs close to the ground at sites of high insect activity. *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. The diet of *T. longimanus* consists of the insect order Diptera, Lepidoptera and Hemiptera (Weterings *et al.*, 2015). Also they are known to prefer cockroaches and beetles (Bates *et al.*, 2008). The evolution of different foraging strategies, corresponding echolocation signals and adaptations of wing morphologies led to differences in the occupied feeding niche which may facilitate the coexistence of sympatric bats (Kusch and Schotte, 2007).

Coexisting species frequently differ in resource use in at least one niche dimension. In sympatric bat species that lived in the same cave or the related species have to have different in some niche dimension to avoid strong competition and thus to facilitate existence in the same area; some sympatric insectivorous bat species change their foraging strategies to another mode (e.g. they change feeding mode between aerial hawking and gleaning) to lower the potential for competition or to reduce interspecific competition between sympatric species, diets, prey selection (Hooper and brown, 1968, Jiang *et al.*, 2008). It was shown in two sympatric and morphologically similar bat species *Rhinolophus affinis* and *R. pearsoni* that lived in the same cave but had differences in echolocation calls that they have a high degree of overlap in group size and also the size of consumed prey (Jiang *et al.*, 2008). Another study conducted on 13 sympatric African insectivorous bats during the dry season showed a broad overlap in all measured niche parameters such as activity pattern, habitat use, and prey selection (Fenton and Thomas, 1980). During the wet season, some insectivorous bat species relied more on beetles than on moths as food. (Fenton and Thomas, 1980). In bats, niche partitioning may be caused by morphological trait differences such as in *Rhinolophus perditus, Hipposideros turpis and Miniopterus fuscus* from Japan based on fecal analyses. These three bats exhibited difference in preferred prey. (Fukai *et al.*, 2009).

1.2.3   Using carbon and nitrogen stable isotope in feeding ecology

Isotopes are atoms with the same number of protons and electrons but differing numbers of neutrons. Stable isotopes are defined as stable since they are not radioactive. Stable isotopes are abundant, the lightest stable isotope account mare than 95% of all isotopes for elements such as hydrogen, carbon, nitrogen, oxygen and sulfur. In the study of ecology, stable isotopes can be used to resolve many problems associated with alternative methods. For example, stable isotope analysis is a useful tool in reconstructing diets, characterizing trophic relationships, elucidating patterns of resource allocation, and constructing food webs (Boecklen *et al.*, 2011).

It has been mostly used as a widespread tool in mammalian ecology which can be particularly in the characterization of community structure, such as dimensions of resource partitioning within species assemblages or nutrient cycles in ecosystem (Crawford *et al.*, 2008) . Stable isotope analysis can be used for several ecological research including elemental circulation in the biosphere, landscape ecology and isotope maps, community ecology and invasive species in food webs and life history ecology and animal migration (Fry, 2006).

Stable isotopes in animal tissues allow the interpretation of its diet, as "You are what you eat". In order to assume the diet of animal based on stable isotope analysis, it is essential to know how stable isotope ratios in consumer tissues are related to dietary values (Hobson and Clark, 1992). Most studies in feeding ecology used carbon and nitrogen isotope ratios to assess habitat use and food webs (Forero ad Hobson, 2003). The beneficial of carbon and nitrogen isotope ratios for study trophic ecology is derived from two properties. First, some sources of diet have distinct isotope signatures, and second, the isotope signature of food is incorporated in the consumer's tissues (Kelly, 2000). The study of stable isotope signatures of bat has confirmed niche partitioning and has provided a guide to the foraging habitats and trophic levels used by bat species (Bullen and Dunlop, 2012) . Carbon and nitrogen isotopic compositions of consumer tissue are thus a function of $\delta^{13}C$ and $\delta^{15}N$ values of consumed prey items; the relative proportions of each prey species assimilated; the isotopic fractionation associated with converting prey tissue into consumer tissue; and in certain instances, foraging location (Bearhop *et al.*, 2004).

The physical and biological processes lead to variations in isotopic abundance in plants and animals (Gannes *et al.*, 1998). $\delta^{13}C$ values can be used to discriminate carbon sources between marine and terrestrial origins (Fry, 2006). Since the heavy isotope of nitrogen is preferentially incorporated into the tissues of consumer from the diet, $\delta^{15}N$ values have been used primarily to assess the position of animal in the food chains, the so-called trophic position. $\delta^{13}C$ values differed among species that relied on C_3, C_4 or CAM; terrestrial C_3 plants, the value of $\delta^{13}C = -27$ ‰ (range -35 ‰ to -21 ‰) and terrestrial C_4 plants, the value of $\delta^{13}C = -13$ ‰ (range -14 ‰ to -10 ‰) (Kelly, 2000). In most natural materials, $\delta^{15}N$ values range between -20 and +45 ‰, while in terrestrial plants is in the range -8 to +18 ‰ (Kelly, 2000). Isotopic discrimination (the difference in isotopic ratios between consumer tissue and diet) during trophic level transfers is often small in carbon isotopes ($\Delta C = 0 - 1$‰), therefore neglected, and then primarily reflect the isotopic ratio of their resource. However, ΔC is not always small. For nitrogen isotope values (ΔN) = 2 - 4 ‰ relative to their diet with each increasing trophic level (Middelburg, 2014). Tissues have specific turnover rates (the time that it takes for isotopes to be replaced by others in a consumer's tissue).

$\delta^{13}C$ values in tissues were ranked in following order of decreasing rate in the different tissues of the gerbil; hair, brain, muscle, liver and fat, respectively (Tieszen *et al.*, 1983). The turnover rates of carbon isotopes in nectar-feeding bat species differed between the three types of tissues in the following order of decreasing turnover rates; blood, wing membrane and hair, respectively (Voigt *et al.*, 2003). In the study of Florida manatee, the mean half-life for $\delta^{13}C$ turnover was 53 and 59 days in the skin form coastal and riverine area, respectively. The mean half-life for $\delta^{15}N$ turnover was 27 and 58 days, respectively (Alves-Stanley and Worthy, 2009). Turnover rates and discrimination factors of $\delta^{15}N$ for seven tissues including plasma, liver, fin, mucus, red blood cells, muscle, and scales. Among tissues, diet-tissue $\delta^{15}N$ discrimination factors ranged from 1.3 to 3.4 ‰. Tissue turnover half-lives ranged from 9.0 to 27.7 days (Heady and Moore, 2013). Stable isotope analysis is also used to assess the diet of birds by detecting pattern of dietary variation and diet composition from liver and muscle samples in three bird populations, in conjunction with their potential food sources. They found seasonal variation can affect isotopic composition and birds responded to temporal changes in food availability, and age related with isotopic differences due to different age comsumed different diets (Nadjafzadeh *et al.*,

2015). In the study of feeding habits of Old World fruit bats by using stable nitrogen isotope, the authors found that $\delta^{15}N$ values varied seasonally. $\delta^{15}N$ values of bat individuals were mainly in correspondence with the range found in plants but 3 bats showed highly enriched $\delta^{15}N$ value, they suggested it probably missed dietary items with irregularity $\delta^{15}N$ values (Herrera *et al*., 2008).

Habitat usage of bats can be estimated based on echolocation activity, the species are predicted to food resources, and from stable isotope of their fur (Bullen and Dunlop, 2012). Stable isotope of bat fur also used to interpret the current local habitat and trophic relationships between species (Dunlop and Bullen, 2011). For example, the diets of bats from the genus *Carollia* have been studied at different sites by comparing values of $\delta^{15}N$ and $\delta^{13}C$ across species of *Carollia.* The study shows that the three species differed in dietary breadth, foraging habitat, and level of insectivory (York and Billings, 2009). Carbon and nitrogen stable isotope ratios at the base of food webs may also vary spatially, and this is reflected in spatial variability in isotopic composition among food webs (Bearhop *et al*., 2004). The stable isotope signatures of tissues generally reflect the diet over the period during which the tissue was synthesized (Hobson and Clark, 1992).

1.3 Objectives

1.3.1   To determine whether four sympatric insectivorous bats differ in niche.

1.3.2   To investigate main prey of four sympatric insectivorous bat species in dry and wet seasons.

CHAPTER 2

Niche of Sympatric Insectivorous Bats in Thailand Revealed by Carbon and Nitrogen Stable Isotopes

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Large dietary niche overlap of sympatric open-space foraging bats revealed by carbon and nitrogen stable isotopes

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Abstract

Sympatric bats engage in various strategies for dietary niche partitioning such as different microhabitat use, however, no previous study has yet looked at potential dietary niche partitioning in mammals foraging in a space void of any physical structure. Here, we used stable isotope ratios of carbon and nitrogen to investigate if three insectivorous bats of central Thailand, *Chaerephon plicatus, Taphozous melanopogon* and *T. theobaldi,* partition food resources when foraging in the open space of the lower boundaries of the troposphere. We quantified the isotopic dietary niches of these species and compared niche dimensions within the guild of open‑space foraging bats and between this guild and the edge‑foraging bat *Hipposideros larvatus.* Our results showed that stable isotope ratios of bats differed between wet and dry seasons. Consistently, open‑space foraging bat species shared a similar isotopic composition in both seasons, which contrasted that of the edge-space foraging bat *H. larvatus.* Isotopic niche dimensions of open-space foraging bats were smaller than those of the edge-space foraging bat. Based on isotopic data, we inferred that open‑space foraging bats foraged mostly on dipterans which may fly or drift to higher altitudes where these bats hunt. In contrast, *H. larvatus* included mostly beetles from C_4 food webs in their diet, highlighting that this species is an important predator of pest insects of C_4 crops, namely cane sugar and corn. Our study emphasizes that the unstructured aerosphere in which open-space foraging bats hunt insects may promote a large overlap in the diet of these species. We conclude that mechanisms other than trophic niche differentiation, such as the motion capacity of bat species, both in terms of covered

distances and accessed altitudes may facilitate the coexistence of these open-space foraging bats.

2.1 Introduction

Sympatric bat species often differ in resource use in at least one dimension of their multi-dimensional niche space. Trophic niche partitioning is the most common mechanism by which animals divide resources and partition critical resources (Schoener, 1986). It is widely assumed that bats partition available microhabitat and food resources based on their wing morphology and echolocation call characteristics. Indeed, based on these two features bats can be separated roughly into seven guilds: open-space aerial, edge-space aerial, edge-space trawling, narrow-space flutter detecting, narrow-space passive gleaning, narrow-space active gleaning and narrow-space passive/active gleaning foragers (Denzinger and Schnitzler, 2013). Past studies have revealed that local insectivorous bat species, particularly closely related species, may have to differentiate in some niche dimension to reduce competition and thus to facilitate local co-existence (Hooper and Brown, 1968). For example, Voigt and coauthors showed that congeneric rhinolophid bats from the same roost cave differed in their wing morphology which may promote different foraging styles in the same habitat and thus coexistence of the two species (Voigt *et al.*, 2010). In a similar case, Jiang and colleagues suggested that competition may be dampened when spatial niche differentiation may enable bats to forage in different microhabitats at the landscape level, even when species were similar in their echolocation call design (Jiang *et al.*, 2008). In most cases, morphological differences in sympatric bats may lead to sufficient resource partitioning to warrant local coexistence over time (e.g. Fukai *et al.*, 2009).

Several techniques have been used to study the feeding ecology of bats, including analysis of stomach content and fecal matter by visual inspection (Leelapaibul *et al.,* 2005, Srilopan *et al.*, In Press, Srinivasulu and Srinivasulu, 2005, Weterings *et al.*, 2015), radio tracking studies (Bontadina *et al.*, 2001, Castle *et al.*, 2015, Roeleke *et* al., 2017), direct observation (Hickey *et al.*, 1996, Acharya and Fenton, 1999) and stable isotope analysis (Broders *et al.*, 2014, Campbell *et al.*, 2017, Dammhahn and Goodman, 2014, Lam *et al.*, 2013, Rex *et al.*, 2010, Voigt *et al.*, 2016). The data obtained from both foraging observations and stomach contents has inherent biases caused by, e.g., differential digestibility of prey items, which are difficult to overcome (Kelly, 2000). Stable isotope analysis has become a widespread tool in ecological studies and is increasingly used to study animal diets over short and long time scale (Ben-David and Flaherty, 2012). This technique is based on the presumption that stable isotope ratios of an animal closely match

that of its diet (DeNiro and Epstein, 1978, 1981). Stable carbon isotope ratios ($\delta^{13}C$) of plants differ according to the specific photosynthetic pathways used by plants (C_3 vs. C_4 plants) and thus the relevance of corresponding food webs (C_3 or C_4 plant-based food webs) for the feeding behavior of animals can be discerned from $\delta^{13}C$ values in their tissues (e.g. DeNiro and Epstein, 1978, Voigt and Kelm, 2006). Nitrogen isotopic ratios ($\delta^{15}N$) in tissues of animals increase gradually at each trophic level ($\Delta N = 2\text{-}4‰$; DeNiro and Epstein, 1981, Vanderklift and Ponsard, 2003). Therefore, $\delta^{15}N$ values can be used to infer the trophic position of an organism (DeNiro and Epstein, 1981, Rex *et al.*, 2010). Further, when tissue with different isotopic retention times are considered, stable isotope ratios can be used to compare temporal and spatial variations in the diet, food web membership and foraging behavior both within and between species (Cryan *et al.*, 2012, DeNiro and Epstein, 1981, Popa-Lisseanu *et al.*, 2014, Sullivan *et al.*, 2006, Voigt *et al.*, 2016). Combining stable nitrogen and carbon isotope ratios may thus delineate the structure of even complex bat assemblages, particularly in those from tropical and subtropical areas (Dammhahn and Goodman, 2014, 2015, Rex *et al.*, 2010, 2011).

Most previous studies on the assemblage structure of bats have been conducted in species that forage in or around vegetation and most studies have focused on temperate zone or neotropical bat assemblages. Here, we studied the diet of four paleotropical insectivorous bat species that occur in sympatry in central Thailand: Three of these species (*Chaerephon plicatus, Taphozous melanopogon* and *T. theobaldi*) belonged to the guild of open-space foraging bats, and one species (*Hipposideros larvatus*) belonged to the guild of edge-space foraging bats. We expected that stable isotope ratios from wing tissues will reveal guild membership because we expected species to feed on insect prey which is distinct in isotopic composition. Specifically, we used stable carbon and nitrogen isotope ratios to assess interspecific differences in the isotopic composition of the diet and habitat use. Further, we hypothesized that the three species of open-space foraging bats should forage on different prey insects in order to facilitate coexistence in the same habitat. Thus, we predicted that $\delta^{13}C$ and $\delta^{15}N$ values and derived isotopic niche dimensions should be different in these three species. Further, we expected strong differences in the isotopic niches of open-space foraging bats compared to the edge-space foraging bat species, which was used for comparison since *H. larvatus* is expected to forage in and around vegetation.

2.2 Materials and Methods

2.2.1 Study species

Horsefield's leaf-nosed bat (*Hipposideros larvatus*) is a species in the family Hipposideridae. This species is found at elevations ranging from 182 to 860 m above sea level. It roosts in caves and in abandoned mines, rock crevices, mines shafts, pagodas, buildings, and tropical moist forest. Roosts are often shared with other bat species and may count up to several hundred bats (Bates *et al.*, 2008). Most of the hipposiderid bats have wing shapes that are suitable for foraging in cluttered environments. *Hipposideros larvatus* has broad wings, with the studied population has average body mass 21.5 ± 3.6 g and average forearm length of 62.1 ± 2.0 mm (n = 62, N. Ruadreo, unpublished data).

Black-bearded tomb bat (*Taphozous melanopogon*) is a species of the family Emballonuridae. This species prefers hilly and forested areas, with freshwater and roosts in caves. (Csorba *et al.*, 2008). It is roosting in groups of thousand individuals. This species has narrow wings with average body mass 28.6 ± 3.0 g and forearm length 65.3 ± 1.3 mm (n = 44, N. Ruadreo, unpublished data).

Theobald's tomb bat (*Taphozous theobaldi*) is a species of the family Emballonuridae. In Thailand, it is the largest representative of the genus (Lekagul and McNeely, 1977). This species usually roosts in caves (Bates *et al.*, 2008). Their average body mass is 37.9 ± 3.6 g and their forearm length is 73.2 ± 1.8 mm (n = 40, N. Ruadreo, unpublished data). Similar to other *Taphozous*, this species has narrow wings and was reported to forage at high altitude up to 800 meters (Roeleke *et al.,* 2017).

The wrinkle-lipped free-tailed bat (*Chaerephon plicatus*) is a species within the family Molossidae. Mollosid bat usually forages at high altitudes, i.e. up to several kilometers, and as far as 25 kilometers from its roost (William *et al.*, 1973). This species forms large colonies of thousands of bats in caves. Its body mass is 15.6 ± 1.8 g and forearm length is 47.1 ± 1.1 mm (n = 42, N. Ruadreo, unpublished data). Their narrow wings with wing loading in male of $0.21 – 0.29$ and in female of $0.20 – 0.29$ g/cm^2 (Leelapaibul *et. al.*, 2003) makes them fast-flying species and hunting in open space such as forested area and over rice fields.

2.2.2   Study sites and sample collection

We conducted our fieldwork in early May and between late July and early August 2016 representing the dry and wet season, respectively. Bats were captured at three caves, 10-12 km to each other, in the Lopburi province, central Thailand, including Wat Khao Tambon cave (15°14' N, 101°16' E) (*H. larvatus* and *T. melanopogon*), Wat Tham Petch Nakha (15°08' N, 101°17' E) (*H. larvatus*) and Khao Wong cave (15°02' N, 101°18' E) (*T. theobaldi* and *C. plicatus*) (Fig. 1). In this area, annual rainfall is 1,140 mm, with 3 seasons consisting of wet, dry-cool and dry, influenced by monsoon wind. The southwest monsoon starts in mid-May and ends in mid-October causing abundant rain especially during August and September. While the northeast monsoon prevails over this area in mid-October to mid-February causing dry-cool weather. Pre-monsoon is the transitional period (mid-February to mid-May) from the northeast to southwest monsoons and the weather becomes dry and hot (Bua Chum meteorological station, 2016). There is usually no rain for at least 5 months.

Within a 30 km radius around caves, there are two major land use types: agricultural crops (52%) and deciduous forest (48%). The agricultural landscape was dominated by two isotopically distinct crop types: C_4 crops (sugar cane (25%) and corn (6%)) and C_3 crops (rice (12%) and cassava (9%)). Each crop is grown at different times of the year. Corn is grown twice a year, yet predominantly during the wet season. Sugar cane is planted in March to June and then harvested about a year later. Cassava is cultivated all year round, yet it mostly grows during the early rainy season. Rice is planted in June or July and harvested from November to December.

In total, we captured 188 insectivorous bats (62 *H. larvatus*, 42 *C. plicatus*, 44 *T. melanopogon* and 40 *T. theobaldi*) with mist nets when bats emerged from the cave. From each individual, we obtained basic information such as sex, body mass (g), reproductive stage, forearm length (mm). Fur samples were collected by gently cutting a small tuft of hair from the interscapular region using small scissors. Fur samples were transferred into 1.5 ml plastic vials and stored in a dry place. Further, we collected wing tissue biopsies from both membranes of the left and right wing using biopsy punches (diameter 3 mm). Wing tissue samples were dried and then stored in 1.5 ml plastic vial. After sample collection, all bats were released at the site of capture.

At night (between 1800h and 0600h), we collected potential insect prey species of bats including Coleoptera, Diptera, Hemiptera and Lepidoptera (Kunz *et al.*, 1995) using modified light traps, consisting of a UV light trap equipped with a fan that sucked insects into the detergent solution container. Modified light traps were set around the caves in selected habitat (rice paddies, sugar cane fields, cassava plantations and forest vegetation), within 10-25 km from the caves. All insects were separated according to trap site and stored in vials with 70% ethanol at room temperature. Samples were then dried at 50°C for 48 hours and kept dry for analyses of carbon and nitrogen stable isotope ratios.
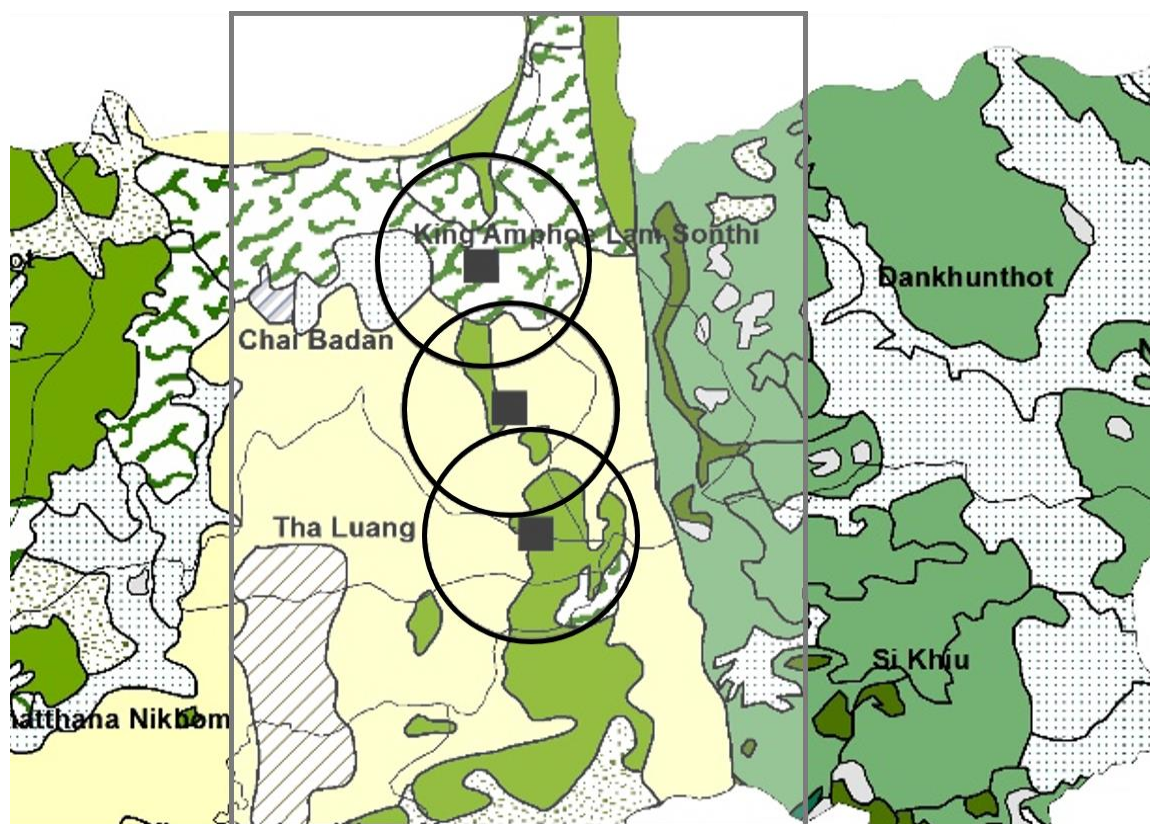
FIG. 1. Location of the study sites at Lopburi Province including KhaoTambon cave, Phet Nakha Cave and KhaoWong Cave. The 10 (square) and 30 (circle) kilometers radius around each cave were indicated.

2.2.3 Stable isotopes analyses

Prior to analysis, all samples were treated with 2:1 chloroform: methanol solution (v/v) for 24 hours to remove lipids and external contaminants. Insect specimens were grounnd to small pieces. In larger insects, we selected thorax for isotopic analysis, assuming that this body part does not deviate significantly from others with respect to isotopic composition. Afterward, samples were dried up 24 h in a drying oven at 50°C. We then used a high-precision balance to transfer 0.5 mg of each sample into a tin capsule. All samples were analyzed by using a Flash EA 1112 Series element analyzer connected in sequence via a ConFlo to a Delta V Advantage isotope ratio mass spectrometer (all ThermoScientific, Bremen Germany). Values are reported in the δ^{13}C and δ^{15}N notation as parts per mille (‰) deviation from the international standard V-PDB for carbon and atmospheric nitrogen for nitrogen. The precision of analysis was better than 0.15‰ for both stable carbon and nitrogen isotope ratios.

2.2.4 Data analyses and statistical analyses

All statistical analyses were performed with R 3.3.4 (R core development group 2014). We tested for seasonal and sexual variations of δ^{13}C and δ^{15}N values in bat tissues using three-way analysis of variance (three-way ANOVA). We compared isotopic niche dimensions across the four study species based on ellipsoids for δ^{13}C and δ^{15}N values calculated with the R-package SIBER (Jackson et al. 2011, Parnell and Jackson 2013). The relative proportion of insect groups in the diet of the studied bats was calculated and plotted using the mixing models from the R-package MixSIAR. To estimate the diet proportion, trophic discrimination factors were applied to the values of potential insects prey species by adding +1‰ for C (DeNiro and Epstein 1987) and +3‰ for N (DeNiro and Epstein 1981) to the corresponding values. All values are presented as means ± one standard deviation.

2.3 Results

### 2.3.1 Isotopic differences beween species, seasons and sex

$\delta^{13}C$ and $\delta^{15}N$ values of fur differed across species ($F_{3,174} = 184.14$, $p < 0.001$, $\delta^{15}N$; $F_{3,174} = 21.54$, $p < 0.001$), but not between seasons ($\delta^{13}C$; $F_{1,174} = 2.96$, $p > 0.05$, $\delta^{15}N$; $F_{1,174} = 0.58$, $p > 0.05$) and between sex ($\delta^{13}C$; $F_{1,174} = 0.01$, $p > 0.05$, $\delta^{15}N$; $F_{1,174} = 1.37$, $p > 0.05$). For all pair-wise comparison across species, we observed significant differences in $\delta^{13}C$ and $\delta^{15}N$ values ($p < 0.05$), except for $\delta^{15}N$ values of *T. theobaldi* and *T. melanopogon* ($p > 0.05$) (Table 1). In wing tissue material, $\delta^{13}C$ and $\delta^{15}N$ values differed across bat species ($\delta^{13}C$; $F_{3,173} = 118.64$, $p < 0.001$, $\delta^{15}N$; $F_{3,173} = 14.63$, $p < 0.001$) and between seasons ($\delta^{13}C$; $F_{1,173} = 102.86$, $p < 0.001$, $\delta^{15}N$; $F_{1,173} = 19.97$, $p < 0.001$), but only $\delta^{13}C$ values differed between sex ($\delta^{13}C$; $F_{1,173} = 5.77$, $p < 0.05$, $\delta^{15}N$; $F_{1,173} = 1.33$, $p > 0.05$). We also observed a significant interaction bewteen species and season ($F_{3,173} = 3.03$, $p < 0.05$) and between species and sex ($F_{3,173} = 6.09$, $p < 0.001$) for $\delta^{13}C$ values. Within species, $\delta^{13}C$ values of wing tissue material were higher during the wet than during the dry season, except for *T. melanopogon* and there were no sex-specific differences in all species for $\delta^{13}C$ values, except *H. larvatus.* During the dry season, $\delta^{13}C$ values of wing tissue remained similar between *T. theobaldi*, *T. melanopogon* and *C. plicatus* ($p > 0.05$), except for *H. larvatus* which differed from all other species ($p < 0.05$). During the wet season, $\delta^{13}C$ values varied between all species, except between *T. melanopogon* and *T. theobaldi* (Fig. 2). $\delta^{15}N$ values ranged between all species except between *H. larvatus* and *T. theobaldi,* and between *T. melanopogon* and *C. plicatus* ($p > 0.05$; Table 2). $\delta^{15}N$ values of wing tissue of *H. larvatus* and *T. melanopogon* were lower in the wet than in the dry season.

Bats consumed insects at varying ratios from both food webs, i.e. food webs based on C_3 and C_4 plants. Based on $\delta^{13}C$ values, we inferred that *H. larvatus* obtained and assimilated similar amounts of carbon from both food webs, while the diet of *T. theobaldi*, *T. melanopogon* and *C. plicatus* was more biased towards the C_3 food web during the dry season. During the wet season, *H. larvatus* consumed more insects from C_4 food webs than C_3 food webs, while *T. theobaldi*, *T. melanopogon* and *C. plicatus* assimilated carbon at similar rates from both food webs. In all four species, $\delta^{13}C$ values were higher during the

wet season than during the dry season, indicating a predominant insect diet from the C_4 food web.

TABLE 1. Stable carbon and nitrogen isotope ratios (mean ± SD) in fur of four sympatric insectivorous bats in the dry and wet seasons, Lopburi, central Thailand. Different superscript letters indicate statistically significant differences at p < 0. 05 (two way ANOVA followed by paired t-test) and n indicates sample sizes.

Species	Dry season			Wet season		
	$\delta^{13}C_{fur}$ (‰)	$\delta^{15}N_{fur}$ (‰)	n	$\delta^{13}C_{fur}$ (‰)	$\delta^{15}N_{fur}$ (‰)	n
H. larvatus[a,e]	-18.11 ± 0.94	9.98 ± 0.92	32	-18.02 ± 1.00	10.07 ± 0.88	30
T theobaldi[b,f]	-21.64 ± 1.01	10.08 ± 0.53	8	-21.07 ± 1.33	10.45 ± 0.50	32
T. melanopogon[c,f]	-22.48 ± 1.16	10.77 ± 0.43	21	-21.86 ± 0.70	10.52 ± 0.44	23
C. plicatus[d,g]	-20.42 ± 0.96	9.80 ± 0.49	17	-20.53 ± 0.95	9.37 ± 0.72	25
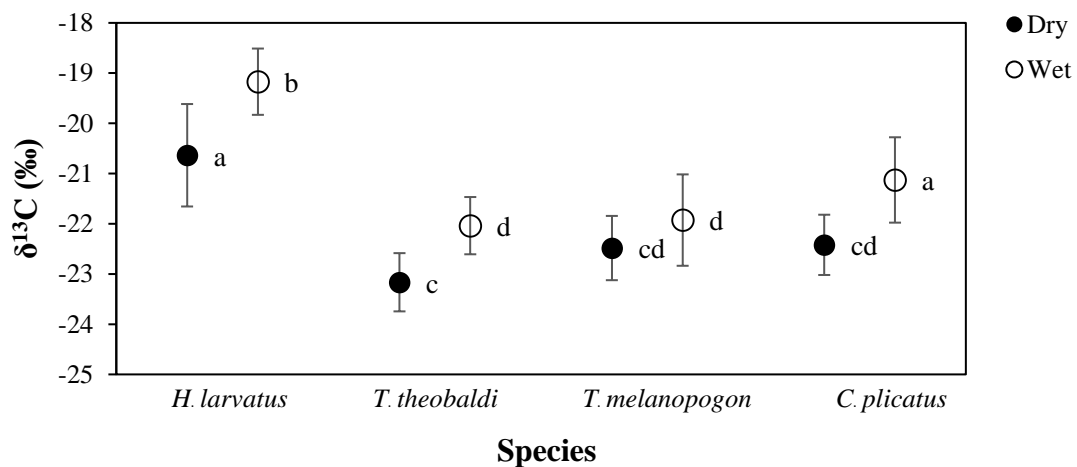
FIG. 2. Average of carbon isotopic ratios (mean ± SD) in wing tissue of four sympatric insectivorous bats in dry and wet seasons, Lopburi, central Thailand. Different letters indicate statistically significant differences at p < 0.05 (two way ANOVA followed by Tukey's test).

TABLE 2. Nitrogen isotopic ratios (mean ± SD) in wing tissue of four sympatric insectivorous bats in the dry and wet seasons, Lopburi, central Thailand. Different superscript letters indicate statistically significant differences at $p < 0.05$ (two way ANOVA followed by paired t-test) and n indicates number of bat individual.

| Species	$\delta^{15}N_{wing}$ (‰)			
	Dry	n	Wet	n
H. larvatus[a]	11.11 ± 0.55	32	10.58 ± 0.72	30
T. theobaldi[a]	10.63 ± 0.19	8	10.68 ± 0.19	32
T. melanopogon[b]	11.19 ± 0.47	21	9.99 ± 0.41	23
C. plicatus[b]	10.58 ± 0.38	17	10.23 ± 0.72	25

2.3.2 Width and overlap of isotopic niches

We estimated the size of the isotopic niches of the four study species by calculating a standardized ellipsoid area (SEA) based on both isotope values obtained from fur material. The ellipsoid area was corrected for small sample sizes (SEA_c). SEA_c decreased in width in the following order; *H. larvatus, T. theobaldi, C. plicatus* and *T. melanopogon*. During the dry season, SEA_c inferred from fur isotopic values was significantly broader for *H. larvatus* than for *T. melanopogon* and *C. plicatus*. Also, SEA_c of *T. theobaldi* was significantly broader than that of *T. melanopogon* in the wet season. SEA_c inferred from wing tissue material was similar between seasons, yet differed across species (sorted according to decreasing values): *H. larvatus, T. melanopogon, C. plicatus* and *T. theobaldi* (Fig. 3). SEA_c from wing tissue of *H. larvatus* was significantly broader than that of *T. theobaldi*, *C. plicatus* and *T. melanopogon* while SEA_c of *T. melanopogon* was significantly broader than that of *T. theobaldi* in the dry season. In the wet season, SEA_c of *H. larvatus* was significantly broarder than that of *T. theobaldi*.

During the dry season, SEA_b of *H. larvatus* and *T. melanopogon* (estimated from fur material) did not overlap with that of other species. During the wet season, SEA_c of *H. larvatus and C. plicatus* did not overlap with those of others species (Fig. 4). For wing tissue, only *H. larvatus* showed no overlap of SEA_c with any of the other species in the dry and wet season. By contrast, SEA_c of *T. theobaldi*, *T. melanopogon* and *C. plicatus* overlapped at various extents (Fig. 5).
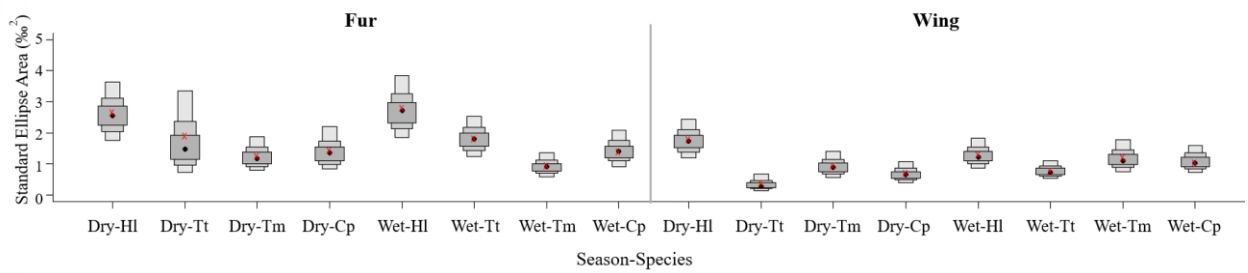
Fig. 3. Standard Ellipses Area (‰^{2}) in fur and wing of four sympatric insectivorous bat species in the dry and wet season (Hl = *H. larvatus*, Tt = *T. theobaldi*, Tm = *T. melanopogon* and Cp = *C. plicatus*). Black dots are the mode SEA, red marks are the sample size-corrected SEA (SEA$_c$), boxes indicate the credible intervals (50% inside dark grey boxes, 75% middle grey boxes, and 95% outer light grey boxes) for Bayesian generated ellipses (SEA) of four sympatric insectivorous.
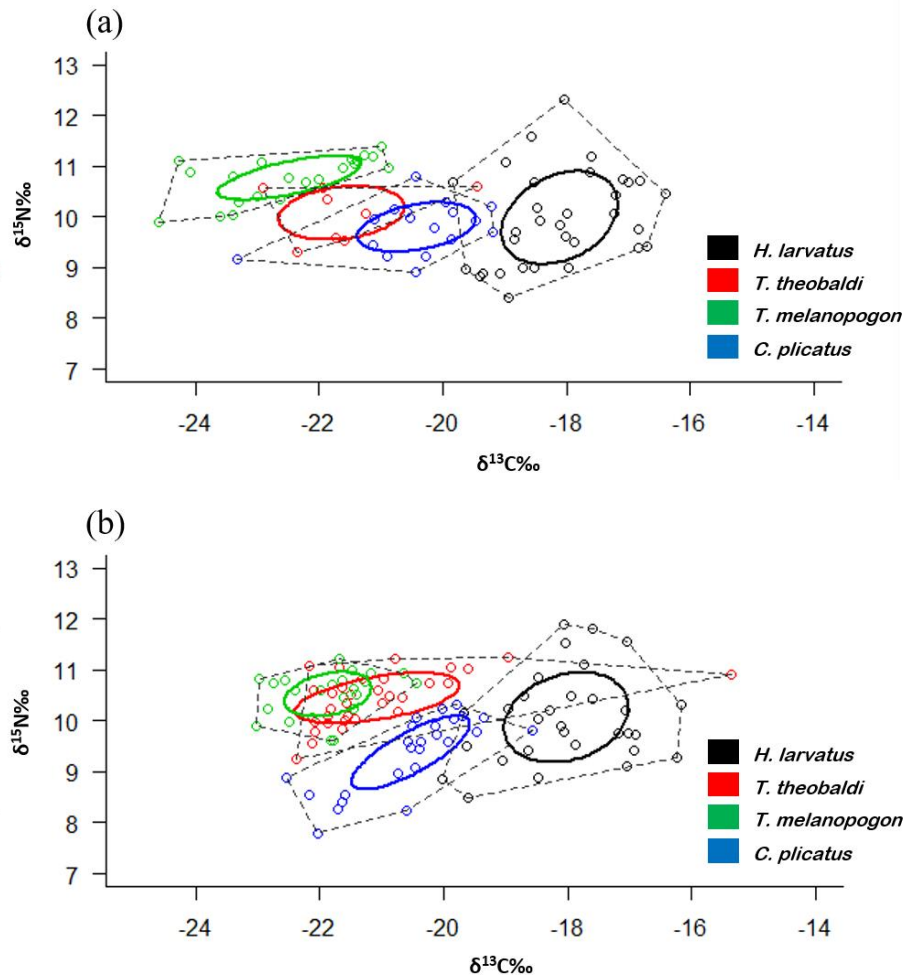
Fig. 4. Bivariate plots of δ^{13}C and δ^{15}N values of fur data collected from four sympatric insectivorous bat species; dashed lines represent convex hull, solid lines represent Standard Ellipses Area (SEA) in the dry (a) and wet (b) season.

(a)

(b)
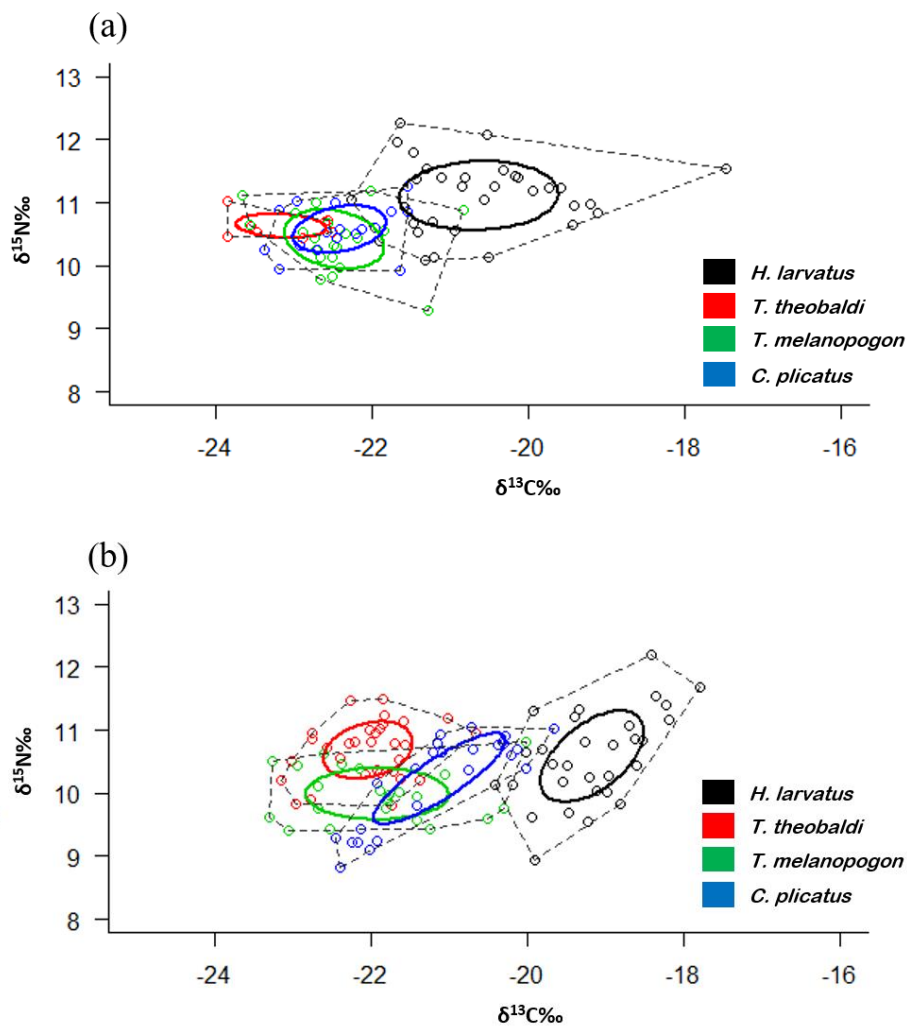
Fig. 5. Bivariate plots of $\delta^{13}C$ and $\delta^{15}N$ values of wing data collected from four sympatric insectivorous bat species; dashed lines represent convex hull, solid lines represent Standard Ellipses Area (SEA) in the dry (a) and wet (b) season.

2.3.3 Consumed insect groups inferred from stable isotope ratios

Overall, mixing models based on stable isotope ratios of wing tissue indicated that all sympatric insectivorous bats differed in the composition of their diet during all seasons. However, in a pair-wise comparison, we observed that the isotopic composition of the diet was similar in *T. theobaldi*, *T. melanopogon* and *C. plicatus*, whereas the isotopic composition of the diet of *H. larvatus* differed from all other studied species.

During the dry season, beetles (Coleoptera) were the primary source of food for *H. larvatus* while dipterans were the main source food for *T. theobaldi, T. melanopogon* and *C. plicatus*. For *H. larvatus* we inferred from mixing models that the diet consisted of the

following insect groups: Coleoptera (48%), Diptera (23%), Lepidoptera (16%) and Hemiptera (13%). For *T. theobaldi* we estimated diets that consisted of insects from the orders Diptera (45%), Lepidoptera (35%), Coleoptera (15%) and Hemiptera (5%). Similarly, *T. melanopogon* had adiet that consisted mostly of Diptera (62%), Lepidoptera (20%), Coleoptera (10%) and Hemiptera (8%). The diet of *C. plicatus* consisted of Dipteran (55%), Lepidopteran (20%), Coleoptera (15%) and Hemiptera (10%). The proportions changed slightly during the wet season. During the wet season, the diet of *H. larvatus* consisted of 43% coleopteran, 32% hemipteran, 15% dipteran and 10% lepidopteran while the diet of *T. theobaldi* consisted of 50% dipteran, 20% lepidopteran, 20% coleopteran and 10% hemipteran. Likewise, the diet of *T. melanopogon* consisted of 67% dipteran, 15% lepidopteran, 10% coleopteran and 8% hemipteran. The diet of *C. plicatus* included 50% dipteran, 20% lepidopteran, 18% coleopteran and 12% hemipteran (Fig. 6, Fig. 7).
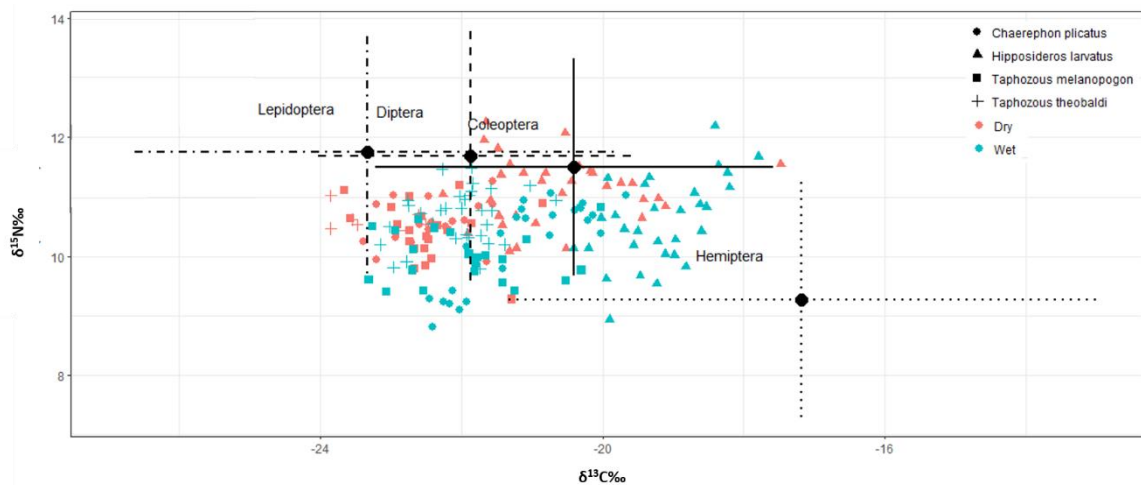
FIG. 6. δ^{13}C and δ^{15}N values of potential prey clutters (mean ± SD) and bat wing tissue from different seasons. Prey clutters were adjusted for Trophic Discrimination Factors (cluster average + TDFs) from literature. We use +1‰ for C (DeNiro and Epstein 1987) and +3‰ for N (DeNiro and Epstein 1981).

(a)

(b)

FIG. 7. The sources of diet contributing to wing tissue of four sympatric insectivorous bats in the dry (a) and wet (b) season.
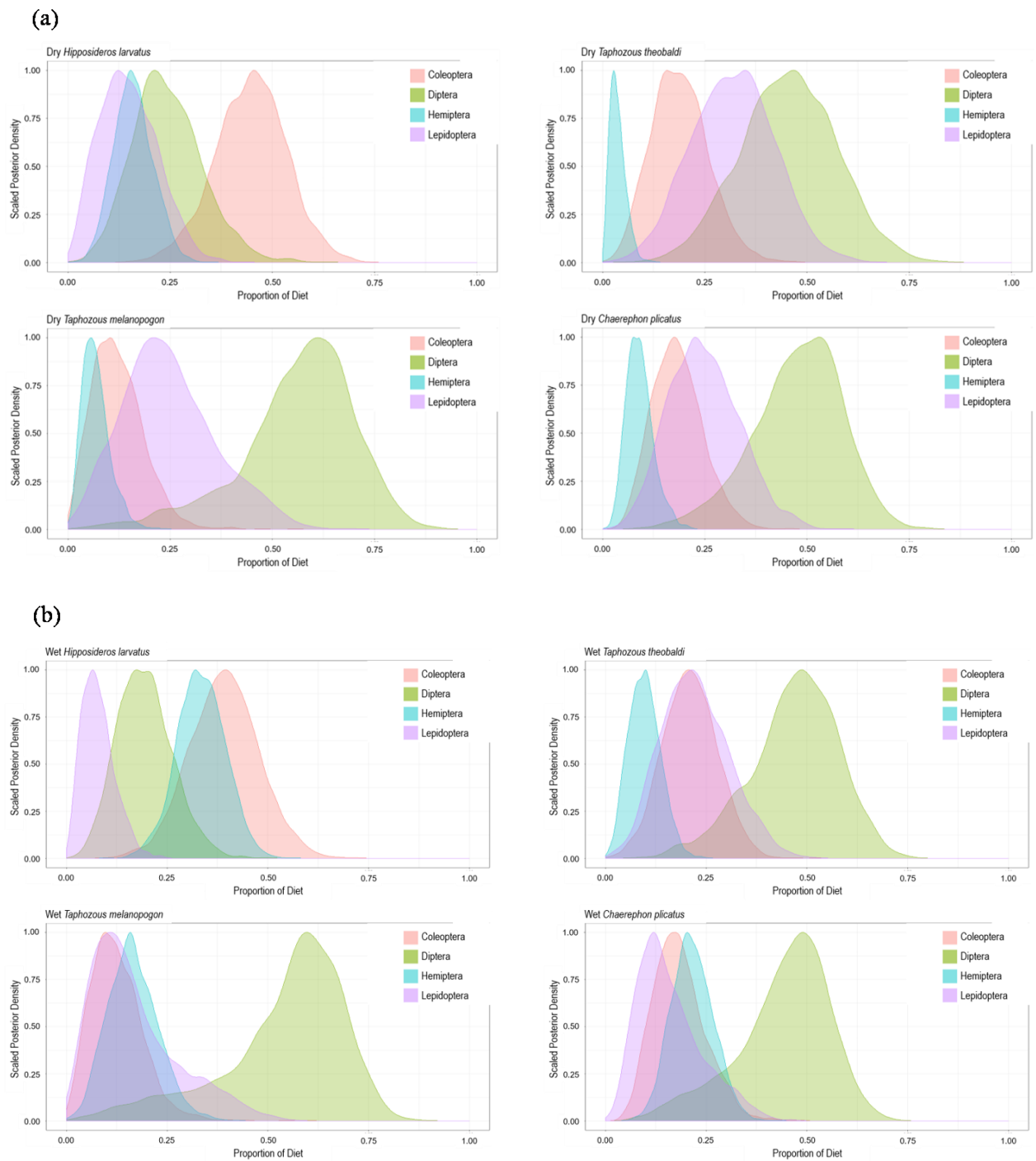
2.4 Discussion

We studied the isotopic dietary niches of four sympatric insectivorous species in central Thailand. In particular, we aimed at shedding light on the niche separation of open-space foraging bat species that lack structural elements in their habitat, i.e. the lower boundaries of the troposphere, which might facilitate the co-existence of species in the same habitat. We found that three open-space foraging bats, *Chaerephon plicatus*, *Taphozous melanopogon* and *T. theobaldi*, share similar isotopic niche spaces when foraging high above the ground for insects. In contrast, edge-space foraging *Hipposideros larvatus* was isotopically distinct from all open-space foraging bats. Furthermore, open-space foraging bats prefers dipterans as their primary dietary source, whereas *H. larvatus* hunts mostly coleopterans.

2.4.1 Seasonal variation in tissues isotopic ratios, sex-specific differences in bat species, niche width and niche overlaping

We found seasonal variations in the isotopic compositions in wing tissue material of bats, but no changes in fur samples. A seasonal variation in the diet of insectivorous bats has already been observed in other species, e.g. in bats of Madagascar (Rakotoarivelo *et al*., 2007). We assumed that the isotopic composition of wing membrane tissue integrates over the period of several weeks prior to sample collection (Voigt *et al.*, 2003, Miron *et al.*, 2016). By contrast, as an inert body product fur integrates over the isotopic composition of the diet during the time of fur growth (Cryan *et al.*, 2004, Fraser *et al.*, 2012). We also found that female *H. larvatus* consumed insects in C_3 food web (forest) higher than male. This may reflect that females forage more than males in the forested area around the caves; especially during the breeding period as found in another study (Bu *et al.*, 2015).

In our study, the roosting caves of *H. larvatus* are located in C_3-dominated areas surrounded by trees and forest vegetation. The observed differences in the isotopic composition of wing tissue material may reflect seasonal changes whereas the isotopic composition of the insect diet may reflect seasonal growth patterns of crop plants and natural vegetation. Increasing $\delta^{13}C$ values might indicate a higher relevance of sugar cane and corn in the diet of bats during the wet season since C_4 plants are more enriched in ^{13}C in relation to ^{12}C than C_3 plants. Usually, the wet season is the period of the year where most farmers plant seedlings and when most of the plant grows. This could support larger

populations of herbivorous insects during the wet season compared to the dry season. Seasonal changes in the relative contribution of insects from C_3 and C_4 food webs may also explain why isotopic niche dimension changes over time. In general, *H. larvatus* exhibited the largest isotopic niche compared to open-space foraging bats, possibly because this species depends more on insects from C_4 food webs. Within a 10 km radius around the cave roost of *H. larvatus*, we found the majority of fields growing sugar cane. Past behavioral studies on hipposiderid species showed that this group of bats forages mostly in the under storey, such as in gap or structure edges with a high flexibility in the specific habitats used (Pavey *et al*., 2001), yet this species group seems to avoid open areas above farmland. Thus, we assume that individuals of *H. larvatus* may have either consumed insects from the C_4 crop plants (sugar cane and corn) at the forest edge structure or they may have even moved along hedgerows or tree rows into crop fields to hunt insects there.

In contrast, aerial insectivorous bats like *Taphozous* and *Chaerephon* mostly forage at high altitude (Roeleke *et al*., 2017, McCracken *et al*., 2008), thus may depend more strongly on a subset of insect prey which has moved to higher altitudes. Accordingly, niche dimensions of these open-space foraging bats were smaller than those of *H. larvatus*. The smaller species of the genus *Taphozous*, i.e. *T. melanopogon,* had a broader niche width than *T. theobaldi*. This might indicate that *T. melanopogon* consumed a larger variety of food insects than *T. theobaldi*. Possibly the larger species is more restricted to the consumption of larger prey insects (Barclay and Brigham, 1991). Furthermore, a larger body size bat may also constrain the aerial maneuverability and that may prevent larger species from hunting small insects (Aldridge and Rautenbach, 1987).

2.4.2   Insect food sources in four sympatric insectivorous bats

The two foraging guilds, i.e. open-space foraging bats (*C. plicatus* and *T. theobaldi* and *T. melanopogon*) and edge-space bats (*H. larvatus*) differed in the insect taxa that they consumed predominantly. The major group of insects ingested by *H. larvatus* was Coleoptera. It is already known that bats that use high duty cycle echolocation such as hipposiderid bats are better able to detect glints from insect wing beats and may therefore detect the echoes of fluttering insects in the cluttered background (Altringham, 2011, Kunz and Fenton, 2003). Furthermore, hipposiderids are known to be flexible in their hunting behavior in being able to prey on airborne insects from perches or by gleaning insects from surfaces. The specific sensory ecology of this taxon makes these bats particularly well

adapted to hunt beetles (Bogdanowicz *et al.*, 1999). Previous studies have already reported that the major food items of hipposiderid bats includes coleopteran, lepidopteran, dipteran and hemipteran insects (Li *et al.*, 2007, Sophia, 2010). Our isotopic data is therefore confirming previous dietary studies in hipposiderid species based on visual inspection of fecal matter.

The stable isotope data of the open-space foraging bats suggested that these species foraged predominantly on dipteran insects and that their dietary niches are large and overlapping. *Taphozous melanopogon*, *T. theobaldi* and *C. plicatus* are in the same foraging mode which is aerial insectivores in uncluttered areas (Altringham, 2011, Bogdanowicz *et al.*, 1999, Kunz and Fenton, 2003, Schnitzler and Kalko, 2001), and therefore, they are likely to encounter a similar set of preys. The specific wing morphology of these genera makes them particularly well adapted to hunt insects in open space (Norberg and Rayner, 1987). Many dipterans generally emerge at dusk and swarm over water bodies or near vegetation structures (Kunz and Fenton, 2003), possibly reaching towards higher altitudes where the open-space foraging bats may have encountered this insect. We assume that many of the studied bats may have hunted swarming dipterans at or closely by Pa Sak water reservoir which is the largest water body within their foraging range (25-30 km). The high percentage of dipterans in the diet of *Taphozous* corresponds well with the study of Weterings and colleagues (2015) who reported a high percentage volume of dipteran in the diet of *T. melanopogon*. However, another study revealed that this bat feeds mainly on insects of the order Coleoptera, Homoptera, and Lepidoptera when inhabiting forested habitat (Srinivasulu and Srinivasulu, 2005). Such contrasting results indicated that this bat is an opportunistic predator with a flexible hunting strategy. Recently, Roeleke *et al.* (2017) revealed that *T. theobaldi* forages at high altitude of up to 550 m by performing undulating altitudinal flights which correspond to the topography of the landscape. We assume that insects that occur at these altitudes are most likely of small size, which corresponds with the diet consisting predominantly of dipterans observed for this species in our study. *Chaerephon plictus* is a specialized aerial-hawking of high-flying insects (Norberg and Rayner, 1987). Possibly, these bats also hunt insects that migrate at higher altitudes. Small dipteran insects are known to be dispersed at high altitudes by wind (Johnson *et al.*, 1962). For example, some fruit flies were found at a range of altitude reaching from ground level to several meters or even more than 1000 m above ground (Johnson *et al.*, 1962, Taylor,

1960a). In addition, coleopteran, homopteran, and lepidopteran are known to migrate at high altitudes. Therefore, we find it likely that migratory insects constitute a major part of the diet in the studied open-space bats. Consistent with this notion, Srilopan *et al.* (in Press) suggested that *C. plicatus* feeds predominantly on migratory planthoppers during the dry season.

Our study highlights that sympatric aerial-hawking insectivorous bats share a diet with similar isotopic composition when foraging in the open space in central Thailand. The diet of *C. plicatus* and the two species of *Taphozous* included mostly dipteran insects, which might be particularly abundant at high altitude owing to their small size and the large potential of getting dispersed by wind drift. The fourth species in our study, *H. larvatus*, shows contrasting isotopic composition and patterns which implies that these batsfeed more heavily on insects from C_4 food webs. Isotopic niche dimensions clearly separated *H. larvatus* from all three open-space foraging bats, which is consistent with the specific foraging habitats of these two feeding guilds. The similarity in the diet of open-space foraging bats raises the question how species such as *C. plicatus*, *T. theobaldi*and*T. melanopogon* can coexist in the same aerial habitat when feeding on similar insect prey. We assume that other niche parameters besides insect prey may separate the niches of these three aerial insectivores. Possibly, they forage at different altitudinal ranges which are constrained by their size, morphology and physiology or they areable to detect different insect groups or size given their species-specific echolocation call design.

2.5 Acknowledgements

2.6 References

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CHAPTER 3

CONCLUSION

Sympatric insectivorous bats adopt several ways to partition their niche. Our results show that each bat species difference in isotopic ratio. Wing tissue showed seasonal variation while not in fur. Based on wing isotopic ratios, four sympatric insectivorous bats altered the dietin different season. Bats fed more on C_4-feeder insects than C_3-feeder insects and tended to feed more on insect herbivores in wet season. Niche width of *Hipposideros larvatus* was the broadest than other three species. Niche of four sympatric bats was slightly overlapped in wet season but more overlapped in dry season. Major prey of *H. larvatus* was coleopteran while *T. melanopogon, T. theobaldi* and *C. plicatus* were dipteran. According to the results, this study further suggests that these four bats can coexist in the same foraging range according eco- morphological characteristic and echolocation differences. The different in niche width might be caused by the ability of each bats to access the prey and habitat.

Recommendation for further study

For more precise results of the diet contributing in bat tissues, we need to study more on their Trophic Discrimination Factors (TDFs) in every focal species. Moreover, further study should determine how these aerial insectivorous bats partition their resources.

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