The influence of artificial light on the foraging efficiency and diet of insect eating bats

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Abstract

Artificial light may be altering the interactions between bats and moth prey. Unable to make use of bat evasion strategies around artificial light, eared moths are susceptible to exploitation by syntonic bats (using echolocation frequencies between 20-50 kHz within the hearing range of eared moths). Using a handheld plasma metabolite analyzer, I evaluated the foraging success of syntonic bats and rarer allotonic bats (using echolocation frequencies outside the hearing range of eared moths), in areas with artificial light and in areas of natural darkness. I used microscope diet analysis to determine whether bats were consuming more or fewer moths in areas with artificial light and in areas of natural darkness.

Syntonic bats were more selective for moth prey under lit conditions, likely owing to a reduction in the ability of tympanate moths to evade bats. Moths increased in the diets of generalist syntonic bats (*Pipistrellus hesperidus*) foraging around artificial light sources. Some *P. hesperidus* individuals showed high β-hydroxybutyrate levels around lights, but there was no difference in β-hydroxybutyrate levels between lit and unlit conditions. There is insufficient evidence to reject the null hypothesis that the foraging success of syntonic bats is equivalent in lit vs unlit conditions. The foraging success and diets of allotonic bats, *Rhinolophus capensis*, appear to be negligibly impacted by artificial light on a small scale.

My study emphasizes the need for a mechanistic understanding of the influence of artificial light on the foraging success of bat species. Bat-moth interactions may be influenced by other factors apart from the common assumption that increased refuelling rates will occur in syntonic species foraging on moths around artificial light.

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Chapter 1

Introduction

Humans are rapidly altering natural habitats by using artificial light to illuminate expanding urban areas (Longcore & Rich 2004, Jones et al. 2009). Light pollution has doubled over the last two decades and now affects 18.7 % of Earth's surface area (Gaston et al. 2013, Caudwels et al. 2014, Elvidge et al. 2014). Globally, artificial light pollution continues to increase at an average rate of 6 % per year, and another 1,527,000 km^2 of land area is expected be urbanized by 2030 (Seto et al. 2011). These drastic changes to the natural light regime have occurred suddenly, relative to evolutionary time, and may result in strong selective pressures with unknown effects on ecosystems (van Langevelde et al. 2011).

Despite having the potential to alter biological processes from the organismal to ecosystem level (van Langevelde et al. 2011), artificial light has not received much research attention. In 2013, there were less than 2000 papers in the Web of Science (compared to climate change with >300,000 papers and chemical pollutants with >1 million papers; Boyles et al. 2013). The impacts of artificial light on ecological interactions remains understudied, but it is known that artificial light disrupts biological processes by altering the natural light regime spatially, temporally and spectrally (Longcore & Rich 2004, Gaston et al. 2013). The natural light regime has been relatively consistent at given latitudes over geological time, providing environmental cues through stages of darkness during the night, which many ecological and evolutionary processes are related to (Gaston et al. 2013).

Many nocturnal species rely on the lunar cycle by using on the moon's pattern of polarized ultraviolet light as a subtle cue (i.e. providing a constant bearing during straight line returns to nesting sites; Longcore & Rich 2004). The strong reliance on ultraviolet light as a cue becomes detrimental around artificial light when insects experience reduced visual sensitivity (by 1000-fold in some large moths; van Langevelde et al. 2011, Gaston et al. 2013). Artificial light can interfere with insect trichromatic and tetrachromatic visual systems (acting as nectar guides; Chittka & Menzel 1992), or navigation systems by impairing orientation and diverting insect flight towards ultraviolet light (Warrant & Dacke 2010, van Langeveld et al. 2011, Gaston et al. 2013). Artificial light and "flight-to-light" behaviour is thought to be one of the major reasons (amongst climate change, habitat degradation and pesticide use) for the declining numbers of moths (the major group of nocturnal pollinators; Frank 1988, McGeachie 1989, Longcore & Rich 2004, Fox 2013).

Moth aggregations around artificial light sources increase foraging opportunities for predators like bats (Rydell 1992, Jones & Rydell 1994, Polak et al. 2011), birds and toads (Frank 1988, Rydell 1992), sit-and wait predatory spiders (Heiling 1999) and even diurnal birds (Davies et al. 2013). By disrupting natural predator-prey interactions (Svensson & Rydell 1998, Longcore & Rich 2004, van Langevelde et al. 2011), artificial light has been shown to alter the trophic balance in aquatic ecosystems by inducing variations in predator foraging behaviour and the distribution of associated prey (Perkin et al. 2011). Evidence for artificial light altering trophic interactions in terrestrial ecosystems is limited, but there are some data for bats and their moth prey suggesting that changes in predator-prey interactions can be induced by artificial light (Acharya & Fenton 1999, Longcore & Rich 2004).

Artificial light is likely to affect species of bats differently, depending on the nature of their echolocation call structure and the prey commonly consumed. Artificial light is not only attractive to moths, but changes tympanate moth behaviour by reducing their defensive strategies against bat predators. The long-standing coevolutionary relationship shared between bats and tympanate moths stems began when certain moths evolved tympanate organs able to detect echolocation frequencies between 20-60 kHz which are commonly used by sympatric bat species (termed syntonic because their echolocation call frequencies fall within the hearing range of eared moths; Connor & Corcoran 2012). The unequal availability of tympanate moths as prey for syntonic bats, due to their echolocation frequency, is postulated by the Allotonic Frequency Hypothesis (Schoeman & Jacobs 2011).

In corroboration with the Allotonic Frequency Hypothesis, some bat species – termed allotonic – may have responded by evolving frequency characteristics of their echolocation calls to be outside the hearing range of moths (Jacobs et al. 2008). These frequency ranges should allow allotonic bats predominant access to tympanate moths as a prey resource. Allotonic echolocation call frequencies typically vary from 8 to 20 kHz and 60 to 215 kHz, depending on the species (Schoeman & Jacobs 2003, Jacobs et al. 2008, Russo et al. 2018).

By changing natural light regimes, artificial light should alter the predator prey interactions between bats and tympanate moths, and the competitive interactions amongst moth predators (Longcore & Rich 2004). The evasive strategies of tympanate moths are not likely as useful as a defense against syntonic bats in environments artificially illuminated at night, where moths use diurnal behavioural strategies. Diurnally, moths are only exposed to ultrasound produced by cicadas and crickets, hence adaptively refrain from anti-bat defenses

(stopping flight and diving to the ground) able to disrupt pheromone tracking and reproductive behaviour (Svensson and Rydell 1998). By making use of diurnal behaviournal strategies around lights, moths are subject to higher predation rates by bats (Frank 1988, Svensson and Rydell 1998, Gaston et al. 2013, Minnaar et al. 2015). A reduction in the abundance of moths, induced by higher rates of predation by syntonic bats around artificial light sources, may place allotonic bats specializing in moth prey at risk – ultimately lowering the fitness of these nocturnal species (Svensson and Rydell 1998).

Allotonic bats, which tend to be rarer, are thought to avoid artificial light (Speakman 1991, Rydell 1992, Longcore & Rich 2004, Kuijper et al. 2008, Stone et al. 2009). Tympanate moth defense systems are adversely impacted by artificial light, creating an open prey base where common syntonic bats now have access to moth prey. There is evidence for increases in the abundance of common syntonic bat species (ie *Pipistrellus* spp.; Arlettaz et al. 2000, Polak et al. 2011, Coleman & Barclay 2012), but an overall loss of bat diversity due to the decline of rarer allotonic bat species (i.e. *Rhinolophus* sp.; Arlettaz et al. 2000, Avila-Flores & Fenton 2005). Increases in syntonic bat species have been correlated with a decrease in allotonic species (Arlettaz et al. 2000, Avila-Flores & Fenton 2005), however this is inconclusive of a cause and effect relationship. Some syntonic bats appear to be benefitting from areas with artificial light (this is not the case for all syntonic species i.e. *Myotis species*; Russo et al. 2017). In contrast, rarer allotonic bats seem to be doing poorly. A possible driving mechanism may be that some syntonic bats are experiencing large increases in foraging success in areas with artificial light, in contrast to allotonic bats which do not. Differences in foraging success rates are associated with shifts in prey selection under artificial light (Minnaar et al. 2015).

My study aimed to address changes to the trophic interactions between bats and insect prey species, in naturally dark areas after the introduction of artificial light, as a potential mechanism explaining the decline in allotonic bat species. This aim is addressed in two data chapters:

- In Chapter two I determined artificial light induced dietary shifts in common bat species with a focus on moth prey. I measured dietary insect proportions, in relation to available prey, consumed by bats in experimentally lit versus naturally dark experimental sites to detect changes in prey selection.
- In Chapter three I evaluated the foraging success of allotonic and syntonic bats in lit and unlit experimental sites. I hypothesized that syntonic bats would have a higher foraging success in lit conditions (relative to unlit conditions), where they were expected to have increased foraging opportunities due to the resulting moth aggregations (Rydell 1992, Minnaar et al. 2015). In contrast, I hypothesized that allotonic bats would have better foraging success foraging in unlit conditions (relative to lit conditions), perhaps as a result of reduced competition or light avoidance behaviour. I used plasma metabolite analysis as a proxy for foraging success as it is a reliable indicator of short-term changes in energy intake (McGuire et al. 2009a, McGuire et al. 2009b, Boyles et al. 2016).

References

ACHARYA, L. & FENTON, M.B. 1999. Bat attacks and moth defensive behaviour around streetlights. *Canadian Journal of Zoology* **77**: 27-33.

ARLETTAZ, R., GODAT, S. & MEYER, H. 2000. Competition for food by expanding pipistrelle bat populations (*Pipistrellus pipistrellus*) might contribute to the decline of lesser horseshoe bats (*Rhinolophus hipposideros*). *Biological Conservation* **93**: 55-60.

AVILA-FLORES, R. & FENTON, M.B. 2005. Use of spatial features by foraging insectivorous bats in a large urban landscape. *Journal of Mammalogy* **86**: 1193-1204.

BARCLAY, M.R. & BRIGHAM, M. 1994. Constraints on optimal foraging: a field test of prey discrimination by echolocating insectivorous bats. *Animal Behaviour* **48**: 1031-1021.

BOYLES, J.G., SOLE, C.L., CRYAN, P.M. & MCCRACKEN, G.F. 2013. On estimating the economic value of insectivorous bats: Prospects and priorities for biologists. In: Adams, R.A., Pedersen, S.C. (Eds.), *Bat Evolution, Ecology, and Conservation*. Spring Science, New York, pp. 501-515.

BOYLES, J.G., MCGUIRE, L.P., BOYLES, E., REIMER, J.P., BROOKS, C.A.C., RUTHERFORD, R.W., RUTHERFORD, T.A., WHITAKER, J.O. & MCCRACKEN, G.F. 2016. Physiological and behavioral adaptations in bats living at high latitudes. *Physiology and Behavior* **165**: 322-327.

CHITTKA, L. & MENZEL, R. 1992. The evolutionary adaptation of flower colours and the insect pollinators' colour vision. *Journal of Comparative Physiology A* **171**: 171-181.

CINZANO, P., FALCHI, F. & ELVIDGE, C.D. 2001. The first world atlas of the artificial night sky brightness. *Monthly Notices of the Royal Astronomical Society* **328**: 689-707.

COLEMAN, J.L. & BARCLAY, R.M.R. 2012. Urbanization and the abundance and diversity of Prairie bats. *Urban Ecosystems* **15**: 87-102.

CONNOR, W. E. & CORCORAN, A.J. 2012. Sound strategies: the 65-million-year-old battle between bats and insects. *Annual Review of Entomology* **57**:21-39.

DAVIES, T.W., BENNIE, J., INGER, R., DE IBARRA, N.H. & GASTON, K.J. 2013. Artificial light pollution: Are shifting spectral signatures changing the balance of species interactions? *Global Change Biology* **19**: 1417-1423.

ELVIDGE, C., HSU, F.C., BAUGH, K.E. & GHOSH, T. 2014. National trends in satellite observed lighting: 1992-2012. In: Weng, Q. (Ed.), *Global Urban Monitoring and through Earth Observation*. CRC Press, Boca Raton, Florida, pp. 97-120.

FRANK, K.D. 1988. Impact of outdoor lighting on moths: an assessment. *Journal of the Lepidopterists' Society* **42**: 63-93.

FOX, R. 2013. The decline of moths in Great Britain: A review of possible causes. *Insect Conservation and Diversity* **6**: 5-19.

GASTON, K.J., BENNIE, J., DAVIES, T.W. & HOPKINS, J. 2013. The ecological impacts of nighttime light pollution: A mechanistic appraisal. *Biological Reviews* **88**: 912-927.

GHANEM, S.J. & VOIGHT, C.C. 2012. Increasing awareness of ecosystem services provided by bats. *Advances in the Study of Behaviour* **44**: 279-302.

HEILING, A.M. 1999. Why do nocturnal orb-web spiders (Araneidae) search for light? *Behavioural Ecology and Sociobiology* **46**: 43-49.

JACOBS, D.S., RATCLIFFE, J.M. & FULLARD, J.H. 2008. Beware of bats, beware of birds: The auditory responses of eared moths to bat and bird predation. *Behavioural Ecology* **19**: 1333-1342.

JONES, G., JACOBS, D.S., KUNZ, T.H., WILIG, M.R. & RACEY, P.A. 2009. Carpe noctem: The importance of bats as bioindicators. *Endangered Species Research* **8**: 93-115.

JONES, G. & RYDELL, J. 1994. Foraging strategy and predation risk as factors influencing emergence time in echolocating bats. *Philosophical Transactions of the Royal Society B* **346**: 445-455.

KUIJPER, D.P.J., SCHUT, J., VAN DULLEMEN, D., TOORMAN, H., GOOSSENS, N., OUWEHAND, J. & LIMPENS, H.J.G.A. 2008. Experimental evidence of light disturbance along the commuting routes of pond bats (*Myotis dasycneme*). *Lutra* **51**: 37-49.

LONGCORE, T. & RICH, C. 2004. Ecological light pollution. *Frontiers in Ecology and the Environment* **2**: 191-198.

MCGEACHIE, W.J. 1989. The effects of moonlight illuminance, temperature and wind speed on light-trap catches of moths. *Bulletin of Entomological Research* **79**: 185-192.

MCGUIRE, L.P., FENTON, M.B., FAURE, P.A. & GUGLIELMO, C.G. 2009a. Determining feeding state and rate of mass change in insectivorous bats using plasma metabolite analysis. *Physiological and Biochemical Zoology* **82**: 812-818.

MCGUIRE, L.P., FENTON, M.B. & GUGLIELMO, C.G. 2009b. Effect of age on energy storage during prehibernation swarming in little brown bats (*Myotis lucifugus*). *Canadian Journal of Zoology* **87**: 515-519.

MINNAAR, C., BOYLES, J.G., MINNAAR, I.A., SOLE, C.L. & MCKECHNIE, A.E. 2015. Stacking the odds: Light pollution may shift the balance in an ancient predator-prey arms race. *Journal of Applied Ecology* **52**: 522-531.

PERKIN, E.K., HOLKER, F. & TOCKNER, K. 2014. The effects of artificial lighting on adult aquatic and terrestrial insects. *Freshwater Biology* **59**: 368-377.

POLAK, T., KORINE, C., YAIR, S. & HOLDERIED, M.W. 2011. Differential effects of artificial lighting on flight and foraging behaviour of two sympatric bat species in a desert. *Journal of Zoology* **285**:21-27.

RYDELL, J. 1992. Exploitation of insects around streetlamps by bats in Sweden. *Functional Ecology* **6**:

744-750.

RUSSO, D., CISTRONE, L., LIBRALATO, N., KORINE, C., JONES, G., & ANCILLOTTO, L. 2017. Adverse effects of artificial illumination on bat drinking activity. *Animal Conservation* **20**: 492-501.

RUSSO, D., ANCILLOTTO, L. & JONES, G. 2018. Bats are still not birds in the digital era: echolocation call variation and why it matters for bat species identification. *Canadian Journal of Zoology* **96**: 63-78.

SCHOEMAN, M.C. & JACOBS, D.S. 2003. Support for the allotonic frequency hypothesis in an insectivorous bat community. *Oecologia* **134**: 154-162.

SETO, K.C., FRAGKIAS, M., GUNERALP, B. & REILLY, M.K. 2011. A meta-analysis of global urban land expansion. *PLoS ONE* **6**: e23777.

SPEAKMAN, J.R. 1991. Why do insectivorous bats in Britain not fly in daylight more frequently? *Functional ecology* **5**: 518-524.

STONE, E.L., JONES, G. & HARRIS, S. 2009. Street lighting disturbs commuting bats. *Current Biology* **19**: 1123-1127.

SVENSSON, A.M. & RYDELL, J. 1998. Mercury vapour lamps interfere with the bat defense of tympanate moths (Operophtera spp.; Geometridae). *Animal Behaviour* **55**: 223-226.

VAN LANGEVELDE, F., ETTEMA, J.A., DONNERS M., WALLIS DEVRIES, M.F. & GROENEDIJK, D. 2011. Effect of spectral composition of artificial light on the attraction of moths. *Biological Conservation* **144**: 2274-2281.

WARRANT, E. & DACKE, M. 2011. Vision and visual navigation in nocturnal insects. *Annual Review of Entomology* **56**: 239-254.

Chapter 2

Artificial light induced diet shifts in common bat species with a focus on eared moths

Introduction

Prey selection, creating dietary links between trophic levels, is an important ecological process shaping the structure of trophic communities (Schaefer et al. 2008). Prey selection is based on the principle of gaining the greatest energetic benefit from prey at the lowest pursuit cost (MacArthur & Pianka 1966). According to optimal diet theory, active prey selection depends on size selective predation, prey relative abundance and the likelihood of prey encounter rates (Emlen 1966, MacArthur & Pianka 1966, Sih & Christensen 2001). There is support of Optimal Diet Theory in systems where prey lack predator avoidance strategies, but in complex systems, where evasive responses of mobile prey may be the most important prey selection factor affecting prey selection, evidence remains equivocal (as per Optimal Foraging Theory accounting for prey pursuit costs; MacArthur & Pianka 1966, Pyke et al. 1977, Sih & Christensen 2001, Schaefer et al. 2008, Minnaar et al. 2015).

Prey selection influenced by prey evasive responses is best modelled in a simple food web where first order predators, such as insectivorous bats, represent trophic interactions with insect pollinators (Ghanem & Voigt 2012). The diets of insectivorous bats can be predicted by the defensive behaviours of their prey, which are largely attributable to their auditory capabilities (Schoeman & Jacobs 2011, Minnaar et al. 2015). A thin tympanate membrane with attached auditory cells and tracheae (Miller & Surlykke 2001) evolved in four out of the nine nocturnal insect orders commonly consumed by bats (Conner & Corcoran 2012).

Important in respect to bat foraging ecology, tympanate organs evolved several times in Lepidoptera, in the families Noctuidae, Pyralidae, Geometridae and some species of Sphingidae. These moths are ecologically important nocturnal pollinators and predation on moths could have cascading effects on plant reproductive output (Miyake & Yahara 1998, Longcore & Rich 2004). Relative to non-tympanate insects, tympanate moths are 40-60 % more successful at evading bat predation. This has been attributed to the long-standing hypothesis of negative phonotactic flight and aposematic acoustics (i.e. the production of ultrasound to match the hearing of an approaching bat; Rydell 1992, Dunning & Kruger 1995, Rydell et al. 1995, Acharya & Fenton 1999). Tympanate moth defenses prevent exploitation by syntonic bats, using echolocation within frequencies of 20-50 kHz which are well within moth hearing range, while allotonic bats that have shifted the frequency of their echolocation outside of this range have exclusive access to tympanate moth prey (Schoeman & Jacobs 2011). Bats are also known to make use of stealth echolocation (low intensity calls) or passive hearing (weak echolocation signals) to avoid detection by moth prey (Russo et al. 2007, Goerlitz et al. 2010). Bats and their moth prey share a long-standing coevolutionary arms race where the major predictor of bat diets is based on tympanate moth defensive abilities, varying with bat peak echolocation frequency (Schoeman & Jacobs 2011).

The long-standing trophic interaction between insectivorous bats and their prey is potentially altered in the presence of modern anthropogenic artificial light. Some of the first evidence of artificial-light induced changes to terrestrial species interactions was given by Acharya & Fenton (1999). Major declines in moth populations have been attributed to compromised moth defensive behaviour in environments artificially illuminated at night where moths revert to diurnal behavioural strategies (Svensson & Rydell 1998, Fox 2013, Miller & Surlykke 2001).

Diurnally, moths are only exposed to ultrasound produced by cicadas and crickets, hence adaptively refrain from undertaking anti-bat defenses (stopping flight and diving to the ground) able to disrupt pheromone tracking and reproductive behaviour (Svensson and Rydell 1998). The lack of evasive manoeuvres by eared moths has been proposed to be the major predictor of bat diets in lit areas (Minnaar et al. 2015), where bats shift their diets towards moth prey (Acharya & Fenton 1999, Minnaar et al. 2015). As moths aggregate in artificially lit areas, increases in moth consumption by some common syntonic bats may also be detrimental to moth-specializing allotonic bat species (van Langevelde et al. 2011, Minnaar et al. 2015).

Thus, the aim of this study was to determine if there are shifts in prey selection by syntonic and allotonic bats around artificial light, in comparison to natural light conditions. To address bat prey preference, I quantified the diversity (species and relative abundance) of nocturnal insects potentially available to bats in the study area. I quantified insect orders through fecal analysis in the diets of common bat species foraging around artificial light and in natural darkness, respectively. I predicted that syntonic bats would increase moth consumption owing to a reduction in moth defenses around artificial light. I expected that allotonic bats would consume high proportions of moths in both artificial and natural lighting conditions.

Methods

Field work:

Study site and experimental design:

I conducted the study in late spring (August 2017) to summer (March 2018) at two study sites in the Eastern Cape Province of South Africa, namely; Kleinrivier Wilderness area and Table

Farm (*see Figure 2.1*). Kleinrivier Wilderness area is situated in the northern slopes of the Grootwinterhoek Mountains, 70 km NE of Port Elizabeth (33.5673° S, 25.2289° E). The Grootwinterhoek Mountains experience a mild Mediterranean climate with the lower slopes characterized by valley bushveld thicket vegetation, progressing into Fynbos near the peaks (Mucina & Rutherford 2006). The Kleinriver area of the mountain range is characterized by a drier climate with an annual rainfall of 244 mm. Table Farm (33.2500°S, 26.4167° E) is situated 20 km N of Grahamstown along the Zuurberg range, and is comprised of a mosaic of Fynbos, valley bushveld thicket, Nama-Karoo and Savannah vegetation. Table Farm receives an average of 380 mm of annual rainfall (personal communication, R. White, Table Farm).

Figure 2.1: Kleinrivier Wilderness area and Table Farm, situated in the Eastern Cape, South Africa (image obtained from Google Imagery 2018).

To determine whether bats consume more or fewer moth pollinators in areas with artificial light or in natural lighting, I followed the experimental design of Minnaar et al. (2015) – *see Figure 2.2*. I netted for bats in naturally dark areas with artificial lights turned off, alternating with experimental areas with artificial lights turned on. The artificial lights consisted of a LED bulb (HWL 10 W, 220-240 V, Osram, Munich, Germany; powered by battery which I charged from a solar panel) elevated 2 m above the ground. Most insects are known aggregate near

high pressure mercury vapour and white energy efficient LED lamps producing high UV (used increasingly globally and attracting 48 % more insects than high pressure yellow sodium vapour lamps; Eisenbeis 2006, Van Grunsven et al. 2014, Pawson & Bader 2014). I alternated my netting efforts between control and experimental treatments at each site during weekly sampling intervals (weather-permitting). Mist nets were placed in areas to maximize trapping rates, including clearings in vegetation and over still water sources. I conducted 35 nights of trapping in the control treatment sites and 50 nights in the experimental treatment sites. On each night, I set up three mist nets around lights (just outside of the lit/ unlit area) following sunset. I tended to mist nets every 10 minutes. I recorded nightly temperature using a thermometer.

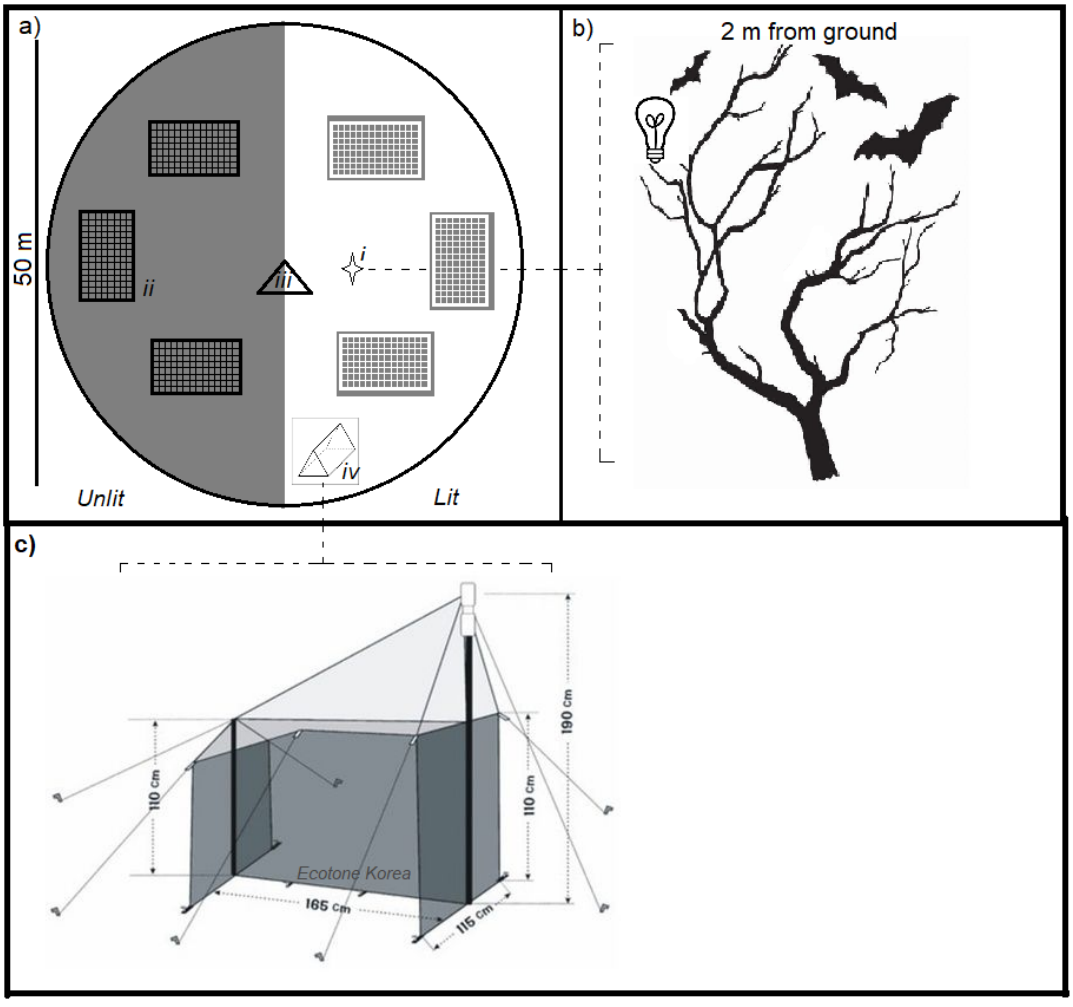


Figure 2.2: Schematic depiction of the experimental set-up (*a*), which followed Minnaar et al. 2015.

Light set-up (*b*) of the LED lamp, elevated in a tree two-meters off the ground, in the artificially lit experiment (*i*). Three mist nets were set up just outside of lit/ unlit area (*ii*) and bat echolocation calls were detected using a Pettersson D100 ultrasound detector to monitor bat activity in the area (*iii*). A Malaise trap (*c*) was set up outside of both lit and unlit areas to sample the nocturnal insect community (*iv*).

Diet sample collection:

I caught a total of 79 bats (45 in the control, 34 in the lit treatment); these include a total of five syntonic species predominantly captured at Kleinrivier (ratios control: dusky pipistrelles *Pipistrellus hesperidus* 18:11, Natal long-fingered bats *Miniopterus natalensis* 2:2, Cape serotines *Neoromicia capensis* 2:2, Egyptian free-tailed bats *Tadarida aegyptiaca* 4:1, and long-tailed serotines *Eptesicus hottentotus* 2:2) and one allotonic species captured at Table Farm (Cape horseshoe bats *Rhinolophus capensis* 18:17; this species was also present at Kleinrivier, but rarely captured). I focused analysis on two species with sufficient sample sizes: allotonic Cape horseshoe bats (hereafter referred to as allotonic bats) and syntonic dusky pipistrelles (hereafter referred to as syntonic bats). The bats were retained individually in cloth bags for one hour until they defecated (based on gut transit times; Buchler 1975). After collecting feces, bats were released on the same night at the site of capture. I froze the fecal pellets for microscopic diet analysis. All aspects of the research were approved by the Rhodes University Ethical Standards Committee (RU-DZE-2018-01-002).

Insect diversity surveys:

To quantify species diversity and relative abundance of nocturnal insects potentially available to bats, I conducted insect surveys to help assess bat prey preference (where prefered taxa are consumed to a greater degree over others; Emlen 1966, Whitaker 1972). I measured aerial insect community composition using a Malaise trap, coupled with UV light attraction, to trap

insects. The Malaise trap is a terylene-netting tent-like structure, funnelling insects after they've flown into the tent wall to a collection jar at the highest point (*see Figure 2.2*). I attached the collection jar and turned UV lights on after sunset and off at sunrise to restrict my sampling to nocturnal insects only. I measured nightly ambient temperature using a thermometer. Insects were stored in a freezer for preservation, after which I identified them to family level and classified moths based on the family-level presence of ears (Scoble 1992). I pooled insect samples per night, calculating the proportional relative abundance for each. Species diversity and richness were calculated using Equation 2.1, Shannon's Diversity Index (*H*; Shannon & Wiener 1949): $H = -\sum_{J=1}^{S} p_i \ln p_i$

Where *S* is the total number of species in the community (species richness) and p_i is the proportion of *S* made up of the *i*th species.

I compared the proportional relative abundance of insect orders comprising the nocturnal insect community to insects in bat diet samples collected on the same night, to determine bat prey preference (Estabrook & Dunham 1976, Burles et al. 2008).

Laboratory work: Microscope analysis

Insect fragments are highly concentrated in bat fecal pellets, as opposed to stomach contents, and provide a means for diet analysis (Kunz & Whitaker 1983, Barclay et al. 1991). While molecular diet analysis allows the accurate identification of small and soft bodied taxa (Clare et al. 2014), this method is costly and soft bodied taxa may be overestimated (Clare et al. 2009). Morphological diet analysis has been established as a reliable method to identify the

insect prey of bats (Barclay et al. 1991, Whitaker et al. 2009) and affordably allows the discrimination between eared versus non-eared prey orders. Microscopic identification of dietary items may result in an overestimate of sclerotized prey, able to survive digestion, as opposed to soft bodied prey (Barclay et al. 1991, Clare et al. 2014). However, studies on the digestive efficiencies of bat prey items indicate that lightly sclerotized insects are identifiable following digestion, owing to the rapid gut transit times of bats (Barclay et al. 1991, Shiel et al. 1997). Considering that some bats may discard moth legs or wings prior to consumption, the digestive efficiency of moth prey ranges from 75-78 % (decreasing for moths with body mass less than 20 mg; Barclay et al. 1991). Being sclerotized and less than 20 mg, the majority of prey is consumed whole by bats and should realistically have in-field digestive efficiencies near 70 % (Barclay et al. 1991). A range of lightly to highly sclerotized prey can be identified through parts or all of wings, scales, legs, antennae and mouth parts (typically mandibles, maxillary palps and labial palps) remaining in bat faecal samples (Barclay et al. 1991, Shiel et al. 1997). Microscopic diet analysis is a well-established method to identify bat prey items, producing results comparable to molecular diet analysis, which is theoretically invulnerable to digestive effects (Whitaker et al. 2009, Goerlitz et al. 2010, Minnaar et al. 2015). Furthermore, when there are digestibility biases of prey items, these will be consistent between unlit and experimentally lit treatments, providing comparable estimates.

I collected fecal pellets from captured bats and pooled diet samples for individual bats (where possible, at least 10 pellets were examined per bat). I moistened fecal pellets by adding drops of glycerine (glycerol) and then separated them into finer pieces using dissecting needles under a dissection microscope (Shiel et al. 1997, Pokhrel & Budha 2015). I separated insect fragments (legs, wings, scales, antennae and mouthparts) from each pellet in a petri-dish.

Fragments were mounted in glycerine to allow viewing under a dissection microscope (10x) and classified to order level using an identification key (Scholtz & Holm 1996, Shiel et al. 1997, Pokhrel & Budha 2015), with the assistance of comparable malaise trap samples collected from the foraging area.

Diet based on fecal analysis is typically expressed as percentage frequency (based on the number of individuals in which a particular food item occurred in their diet) and visual estimates of percentage volume (the numbers of individual insects represented in each pellet). Percentage frequency and percentage volume can be interpreted differently. For example, small insects occurring in low numbers in all pellets of a bat will represent a high percent frequency but low percentage volume (Kunz & Whitaker 1983). Whereas, visually estimating whole insect numbers from insect fragments can result in a high bias for percentage volume estimates (Kunz & Whitaker 1983). To eliminate biases in interpretation, I calculated both percentage frequency and volume to assess differences in the consumption of prey orders around lights versus in darkness. I calculated the percentage frequency of each insect order per individual using Equation 2.2 (Shiel et al. 1997): $\% \, Frequency = 100[\frac{O}{T}]$; where *O* is the number of occurrences of the category i.e. number of droppings containing it and *T* is the total occurrences for all categories. The total percentage volume per individual bat was calculated using the Equation 2.3 (Kunz & Whitaker 1983): $\% \, Volume = 100[\frac{\sum Ci}{Ti}]$; where *Ci* is the individual pellet volumes of category and *Ti* is the total volume of all pellets sampled per individual.

Data analysis:

I used linear models (ANOVA function in R with the package "car" to obtain significance based on F values) to determine the relationship of the composition of bat diets (proportions of each insect order converted to linear data using the logit function) as a function of treatment (lit or unlit). Model covariates included available prey (proportions converted to linear data using the logit function), insect order (Lepidoptera, Coleoptera, Diptera, Hemiptera, Other), echolocation strategy (allotonic or syntonic) and night as a factor (representing seasonal variation). Where ANOVA was significant, I performed a Tukey's post-hoc test to detect which dietary orders consumed differed between allotonic and syntonic species as a function of treatment.

I used a Chi-squared test to assess whether syntonic and allotonic bats showed preferences for foraging on moth prey in each treatment, relative to available moth prey in the community. I calculated the X^2 values for each individual bat, using the observed proportion of moth prey in the diet, and the expected proportion of available moth prey. I used these values to perform a Chi-square distribution test on the sum of X^2 values.

Results

The Kleinrivier site had a mean insect species diversity of 3.33±0.50 (mean ± SD) and richness of 7.30±3.93, in comparison to Table Farm with a mean diversity of 2.59±0.10 and richness of 5.91±1.65. Insect communities at both study sites had high proportions of Lepidoptera and Diptera (*Figure 2.3*). These orders have been shown to dominate in Fynbos and Nama-Karoo insect communities (Schoeman & Jacobs 2003, Schoeman & Jacobs 2011). Variation in the

insect community over time (supplementary *Figure S1*) exhibited no clear pattern in relation to air temperature (r = 0.009, n = 44, p = 0.340). Eared moths represented 73 % and 83 % of Kleinrivier and Table Farm moth communities respectively (supplementary *Table S1*).

Insect orders were consumed by bats in significantly different amounts (t = 1.60, df = 3, p<0.001), where Lepidoptera and Coleoptera constituted the highest prey volume in all bats. Despite being abundant in the insect community, Diptera were not commonly consumed by bats (*Figure 2.4*).

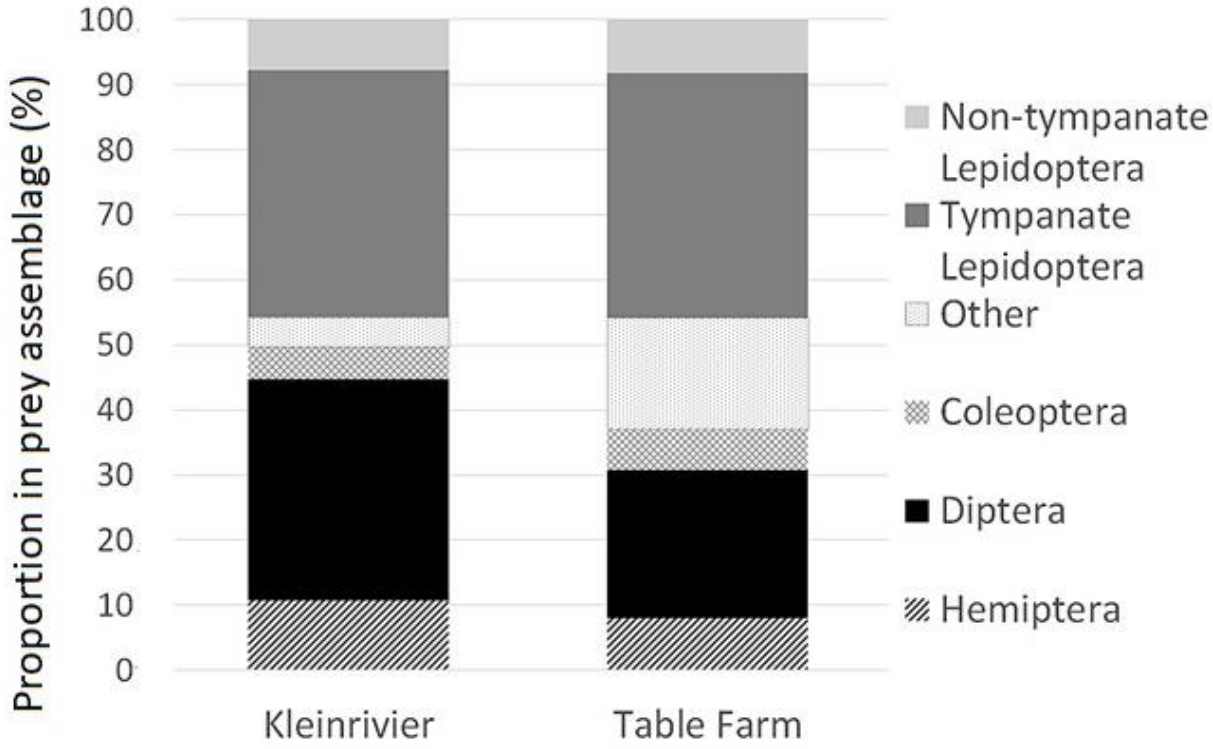


Figure 2.3: The proportion of insects comprising the prey assemblage potentially available to bats. The prey communities at both Kleinrivier and Table Farm were dominated by Lepidoptera and Diptera.

Echolocation (syntonic or allotonic) was a significant (t = 1.60, df = 1, p<0.001) determinant of bat diets, where treatment (lit vs non-lit) was only statistically significant in determining the diet of syntonic dusky pipistrelles. Syntonic pipistrelles consumed significantly more

moths in the lit treatment relative to unlit conditions (t = -0.98, df = 28, p<0.001). Moth proportions consumed by allotonic horseshoe bats did not differ significantly across treatments (t = -1.01, df = 34, p = 1.000, *Figure 2.4*).

Beetle proportions consumed by bats under unlit conditions significantly differed (t = -1.26, df = 55, P<0.001) between syntonic and allotonic bats, where the latter consumed lower proportions of beetles and more moths. However, the proportions of beetles consumed did not differ significantly between syntonic and allotonic bats under lit conditions where some syntonic species decreased beetle consumption. Dietary moth proportions significantly differed (t = 8.63, df = 34, p<0.001) between syntonic and allotonic bats in the unlit treatment, but not in the lit treatment (t = 0.19, df = 17, p = 0.840) where syntonic pipistrelles increased moth consumption (*Figure 2.4*).

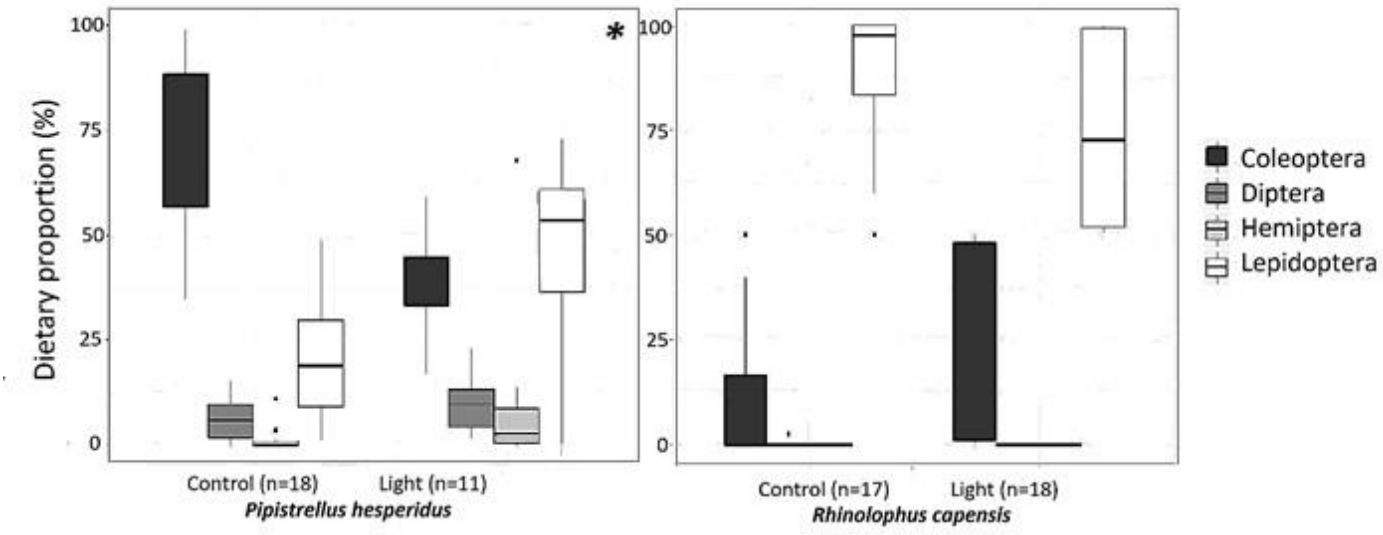


Figure 2.4: Variation in proportion of insect orders consumed by syntonic (*P. hesperidus*) and allotonic (*R. capensis*) species across lit and unlit treatments. Dietary proportions are reflective of percentage volume of dietary constituents. Dusky pipistrelles (*P. hesperidus*) exhibited significant increases in moth consumption under artificial light (*).

Results from the Chi-squared analysis indicated that both syntonic and allotonic bats, in both treatments, consumed moths at significantly different proportions from those expected

based on the nightly moth proportions available in the prey community (t = 8.63, df = 44, p<0.001). Allotonic bats selected significantly more moths from the available prey community in the both the lit and unlit treatment. Syntonic bats consumed fewer moths in the unlit treatment relative to available moth prey. However, in the lit treatment, syntonic bats consumed more moths than expected – thus selecting for moth prey (*Figure 2.5*).

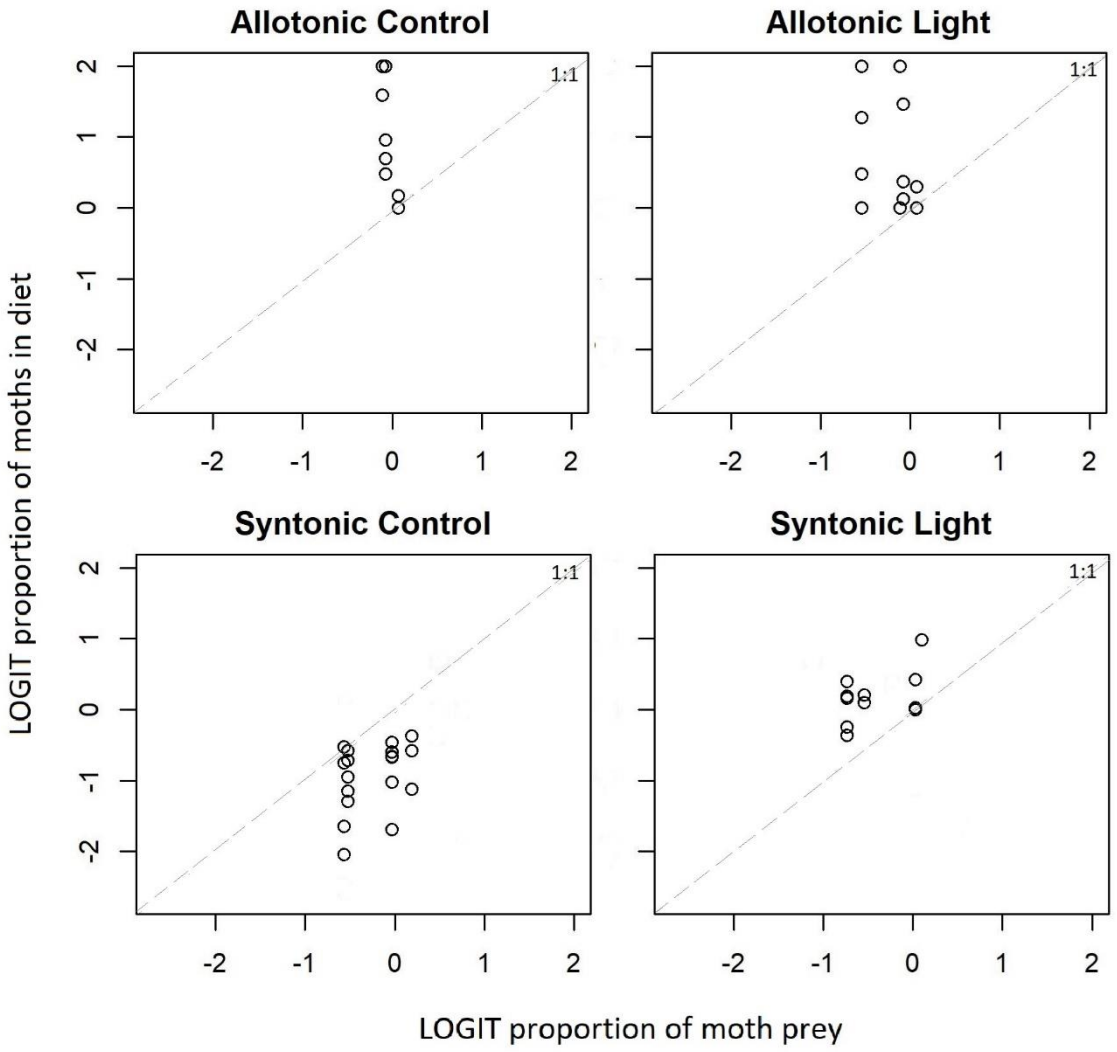


Figure 2.5: Moth prey selection by allotonic and syntonic bats in relation to prey abundance, where the dashed line represents prey eaten in accordance to their abundance (x=y). Bats are assumed to have avoided moth prey when values fall below the line, and select for moth prey for values above the line.

Among the species caught too rarely for quantitative analysis, Natal long-fingered bats consumed a qualitatively high proportion of moths in both treatments. Long-tailed serotines did not consume a high proportion of moths in either treatment, and instead fed mainly on beetles. Both Egyptian free-tailed bats and Cape serotines fed mainly on beetles in unlit conditions. The single Egyptian free-tailed bat captured under lit conditions consumed a high proportion of moths (Supplementary *Figure S2*).

Discussion

I found that syntonic bats increased selection for moth prey in artificially lit conditions. These results were consistent with my hypothesis that syntonic bats would consume more moth prey around artificial light. Allotonic bats selected for moth prey in both treatments, as predicted. I was limited in catching syntonic and allotonic bats from different sample sites respectively, but I argue that treatment differences override habitat differences as both sample sites share similar insect and bat communities (Monadjem et al. 2010). The prey communities at both study sites were dominated by tympanate moths, indicating that prey selection is likely driven by the Allotonic Frequency Hypothesis under natural lighting conditions (Schoeman & Jacobs 2011). This hypothesis postulates that tympanate moth prey are not equally available to syntonic bats. In contrast, syntonic bats significantly increase selection for moth prey around artificial light. This may be attributed to a reduction in the efficiency of eared moth defences. The diets of allotonic Cape horseshoe bats do not appear to be impacted by artificial light. Whereas, increased selection for moth prey by syntonic bats may imply a loss of generalist feeding strategies by some syntonic bats. Generalist feeding is thought to be required to stabilize complex food web interactions (Fenton 1977, Clare et al. 2014).

The highest occurrence of tympanate moth prey occurred in the diets of allotonic horseshoe bats, as predicted by the Allotonic Frequency Hypothesis (Schoeman & Jacobs 2011) under natural lighting conditions. By using high-pitched constant frequency echolocation (at 83.9 kHz; Monadjem 2010), Cape horseshoe bats can avoid detection by tympanate moths (Schoeman & Jacobs 2003, Schoeman & Jacobs 2011). These endemic Cape horseshoe bats consumed high moth proportions in both treatments in my study, however it is known that this species feeds on a seasonally variable diet of Lepidoptera and Coleoptera (Jacobs et al. 2007, Schoeman & Jacobs 2011).

In addition to switching prey items under artificial light, some syntonic species may switch from standard generalist diets to opportunistically feed on moth prey around artificial light sources (Fenton et al. 1977; Rydell 1992; Burles et al. 2008). Under lit conditions, syntonic bats may reduce or avoid consumption of insect orders with lower energetic value (Minnaar et al. 2015). Syntonic dusky pipistrelles, which are generalists known to feed on Hemiptera, Diptera, Neuroptera, and non-eared Lepidoptera (Monadjem et al. 2010), showed the expected increase in dietary specialization around lights. Conversely, it appears that naturally selective syntonic species do not show large shifts in diet around lights, but they might increase selection for their preferred prey (Cravens et al. 2018). Qualitatively, my limited data on other syntonic species in this community support this proposal. Natal long-fingered bats consumed high proportions of moths in both treatments and long-tailed Serotine bats specialized on beetle prey (Schoeman and Jacobs 2003). As the majority of artificial light studies have focused on moth favouring bats (i.e. hoary bats *Lasiurus cinereus*, red bats *L. borealis* and Hawaiian hoary bats *L. cinereus semotus*; Acharya & Fenton 1992, Acharya & Fenton 1999), increased moth consumption around artificial light should not be

assumed for all species. Increases in moth consumption may be species-specific (i.e. *Myotis* species avoid lights; Russo et al. 2017), possibly only occurring in generalist (and in some cases moth-favouring) syntonic species.

By moving away from allotonic bat foraging areas to aggregate around lights, moths are fed on by generalist syntonic bats and the quality of available prey for allotonic species may be reduced (Safi & Siemers 2010, Minnaar et al. 2015). My study did not allow me to directly test the influence of artificial-light on the ecological interactions between endangered endemic Cape horseshoe bats and syntonic dusky pipistrelles at the same site. However, I found that horseshoe bats consumed similar proportions of moth prey in both lit and unlit treatments. Hence, the presence of artificial light over a small spatial and temporal scale may not produce significant changes in the diets of allotonic Cape horseshoe bats. Regardless, Cape horseshoe bats may be able to adapt to reductions in moth prey as this species also feeds on fatty, nutrient-rich beetle prey (Studier & Sevick 1992, Clare et al. 2014). Rather, large scale artificial lighting may negatively impact allotonic bats that avoid light or rely on moths as their sole prey resource, by lacking the skull morphology to consume other prey types or are unable to shift diets for other reasons (Safi & Siemers 2010, Minnaar et al. 2015).

My findings are consistent with the interpretation that artificial lighting conditions allow syntonic bats increased access to moth prey due to a reduction in tympanite moth defences. My results corroborate the findings of Minnaar et al. (2015; however this study used mercury vapour lights) and Cravens et al. (2017), suggesting that irrespective of technology, artificial lighting changes ecological interactions between bats and their prey. Artificial lighting does not appear to significantly impact the diets of bats with adaptations that allow them to specialize on eared moths (allotonic bats that did not exhibit light avoidance behaviour) or

the diets of bats with strong foraging preferences (syntonic specialists). However, bats with generalist diets that cannot avoid detection by eared moths (syntonic generalists) appear to shift their diets in the presence of artificial lighting to take advantage of this newly accessible prey. Generalist feeding stabilizes complex food web interactions (Fenton 1977; Clare et al. 2014) so anthropogenic light sources that impact the foraging decisions of generalist nocturnal insectivores may also influence additional shifts in community composition.

References

ACHARYA, L. & FENTON, M.B. 1999. Bat attacks and moth defensive behaviour around streetlights. *Canadian Journal of Zoology* **77**: 27-33.

ANTHONY, E.L. & KUNZ, T.H. 1977. Feeding strategies of the Little Brown bat, *Myotis lucifugus*, in Southern New Hampshire. *Ecology* **58**: 775-786.

AVILA-FLORES, R. & FENTON, M.B. 2005. Use of spatial features by foraging insectivorous bats in a large urban landscape. *Journal of Mammalogy* **86**: 1193-1204.

BARCLAY, R.M., DOLAN, M.A., & DYCK, A. 1991. The digestive efficiency of insectivorous bats. *Canadian Journal of Zoology* **69**: 1853-1856.

BUCHLER, E.R. 1975. Food transit time in *Myotis lucifugus* Chiroptera: Vespertilionidae. *Journal of Mammalogy* **56**: 252-255.

BURLES, D.W., BRIGHAM, R.M., RING, R.A. & REIMCHEN, T.E. 2008. Diet of two insectivorous bats, *Myotis lucifugus* and *Myotis keenii*, in relation to arthropod abundance in a temperate Pacific Northwest rainforest environment. *Canadian Journal of Zoology* **86**: 1367-1375.

CLARE, E.L., FRASER, E.E., BRAID, H.E., FENTON, M.B. & HEBERT, P.D.N. 2009. Species on the menu of a generalist predator, the eastern red bat (*Lasiurus borealis*): Using a molecular approach to detect arthropod prey. *Molecular Ecology* **18**: 2532-2542.

CLARE, E.L., SYMONDSON, W.O.C. & FENTON, B. 2014. An inordinate fondness for beetles? Variation in seasonal dietary preferences of night-roosting big brown bats (*Eptesicus fuscus*). *Molecular Ecology* **23**: 3633-3647.

CONNER, W.E. & CORCORAN, A.J. 2012. Sound strategies: the 65-million-year-old battle between bats and insects. *Annual Review of Entomology* **57**:21-39.

CRAVENS, Z.M., BROWN, V.A., DIVOLL, T.J., & BOYLES, J.G. 2017 Illuminating prey selection in an

insectivorous bat community, exposed to artificial light at night. *Journal of Applied Ecology* DOI: 10.1111/1365-2664.13036.

DUNNING, D.C. & KRUGER, M. 1995. Aposematic sounds in African moths. *Biotropica* 227-231.

EISENBEIS, G. 2006. Artificial night lighting and insects: attraction of insects to streetlamps in a rural setting in Germany. In: Rich, C., Longcore, T. (Eds.), *Ecological Consequences of Artificial Night Lighting*. Island Press, Washington DC, pp. 281-304.

EMLEN, J.M. 1966. The role of time and energy in food preference. *The American Naturalist* **100**: 611-617.

ESTABROOK, G.F. & DUNHAM, A.E. 1976. Optimal diet as a function of absolute abundance, relative abundance, and relative value of available prey. *The American Naturalist* **110**: 401-413.

FENTON, M.B., BOYLE, N.G.H., HARRISON, T.M. & OXLEY, D.J. 1977. Activity patterns, habitat use and prey selection by South African insectivorous bats. *Biotropica* **9**: 73-85.

FENTON, M.B. & FULLARD, J.H. 1979. The influence of moth hearing on bat echolocation strategies. *Journal of Comparative Physiology A* **132**: 77-86.

FOX, R. 2013. The decline of moths in Great Britain: A review of possible causes. *Insect Conservation and Diversity* **6**: 5-19.

GHANEM, S.J. & VOIGHT, C.C. 2012. Increasing awareness of ecosystem services provided by bats. *Advances in the Study of Behavior* **44**: 279-302.

GOERLITZ, H.R., TER HOFSTEDE, H.M., ZEALE, M.R.K., JONES, G. & HOLDERIED, M.W. 2010. An aerial-hawking bat uses stealth echolocation to counter moth hearing. *Current Biology* **20**: 1568-1572.

JACOBS, D.S., BARCLAY, R.M.R. & WALKER, M.H. 2007. The allometry of echolocation call frequencies of insectivorous bats: why do some species deviate from the pattern? *Oecologia* **152**: 583-594.

JACOBS, D.S., RATCLIFFE, J.M. & FULLARD, J.H. 2008. Beware of bats, beware of birds: The auditory responses of eared moths to bat and bird predation. *Behavioral Ecology* **19**: 1333-1342.

JUNG, K. & KALKO, E.K.V. 2010. Where forest meets urbanization: foraging plasticity of aerial insectivorous bats in an anthropogenically altered environment. *Journal of Mammalogy* **91**: 144-153.

KUNZ, T.H., & WHITAKER, J.O. 1983. An evaluation of fecal analysis for determining food habits of insectivorous bats. *Canadian Journal of Zoology* **61**: 1317-1321.

LONGCORE, T. & RICH, C. 2004. Ecological light pollution. *Frontiers in Ecology and the Environment* **2**: 191-198.

MACARTHUR, R.H. & PIANKA, E.R. 1966. On optimal use of a patchy environment. *The American Naturalist* **100**: 603-609.

MARGALEF, D.R. 1958. Information theory in ecology. *International Journal of General Systems* **3**: 36-71.

MILLER, L.A. & SURLYKKE, A. 2001. How some insects detect and avoid being Eaten by bats: tactics and countertactics of prey and predator. *BioScience* **51**: 570-581.

MINNAAR, C., BOYLES, J.G., MINNAAR, I.A., SOLE, C.L. & MCKECHNIE, A.E. 2015. Stacking the odds: Light pollution may shift the balance in an ancient predator-prey arms race. *Journal of Applied Ecology* **52**: 522-531.

MIYAKE, T. & YAHARA, T. 1998. Why does the flower of *Lonicera japonica* open at dusk? *Canadian Journal of Botany*, **76**: 1806-1811.

MONADJEM, A., TAYLOR, P.J., COTTERILL, W., & SCHOEMAN, M.C. 2010. *Bats of southern and central Africa: a biogeographic & taxonomic synthesis*, Johannesburg: Wits University Press, pp. 596.

NORBERG, U.M., & RAYNER, J.M.V. 1987. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Philosophical Transactions of the Royal Society of London, Biological Sciences* **316**: 335-427.

PAWSON, S.M. & BADER, M.K.F. 2014. LED lighting increases the ecological impact of light pollution irrespective of colour temperature. *Ecological Applications* **24**: 1561-1568.

POKHREL, S., & BUDHA, P.B. 2015. Key to identify insects from droppings of some insectivorous bats of Nepal. *Journal of Institute of Science and Technology* **19**: 129-136.

PYKE, G.H., PULLIAM, H.R., & CHARNOV, E. L. 1977. Optimal foraging: a selective review of theory and tests. *The Quarterly Review of Biology* **52**: 137-154.

RUSSO, D., JONES, G. & ARLETTAZ, R. 2007. Echolocation and passive listening by foraging mouse-eared bats *Myotis myotis* and *M. blythii*. *Journal of Experimental Biology* **210**: 166-176.

RUSSO, D., CISTRONE, L., LIBRALATO, N., KORINE, C., JONES, G., & ANCILLOTTO, L. 2017. Adverse effects of artificial illumination on bat drinking activity. *Animal Conservation* **20**: 492-501.

RYDELL, J. 1992. Exploitation of insects around streetlamps by bats in Sweden. *Functional Ecology* **6**: 744-750.

RYDELL, J., JONES, G. & WATERS, D. 1995. Echolocating bats and hearing moths: who are the

winners? *Oikos* 419-424.

SAFI, K. & SIEMERS, B.M. 2010. Implications of sensory ecology for species coexistence: Bias links predator diversity to prey size distribution. *Evolutionary Ecology* **24**: 703-713.

SCHAEFER, H.M., SPITZER K. & BAIRLEIN, F. 2008. Long- term effects of previous experience nutrient discrimination abilities in birds. *Frontiers in Zoology* **5**: 4.

SCHOEMAN, M.C. 2016. Light pollution at stadiums favors urban exploiter bats. *Animal Conservation* **19**: 120-130.

SCHOEMAN, M.C. & JACOBS, D.S. 2003. Support for the allotonic frequency hypothesis in an insectivorous bat community. *Oecologia* **134**: 154-162.

SCHOEMAN, M.C. & JACOBS, D.S. 2011. The relative influence of competition and prey defenses on the trophic structure of animalivorous bat ensembles. *Oecologia* **166**: 493-506.

SCHOLTZ, C.H. & HOLM, E. 1996. Insects of Southern Africa, 2nd edn (eds C.H. Scholtz & E. Holm). Protea Book House, Pretoria.

SCOBLE, M.J. 1992. The Lepidoptera. Form, Function and Diversity. Oxford University Press, Oxford.

SHANNON, C.E. & WIENER, W., 1949. The mathematical theory of communication. Urbana, University of Illinois Press, 177p.

SHIEL, C., MCANEY, C., & SULLIVAN, C. 1997. Identification of arthropod fragments in bat droppings. The Mammal Society, London.

SIH, A. & CHRISTENSEN, B. 2001. Optimal diet theory: when does it work, and when and why does it fail? *Animal Behaviour* **61**: 379-390.

STUDIER, E.H. & SEVICK, S.H. 1992. Live mass, water content, nitrogen and mineral levels in some insects from south-central lower Michigan. *Comparative Biochemistry and Physiology Part A: Physiology* **103**: 579-595.

SVENSSON, A.M. & RYDELL, J. 1998. Mercury vapour lamps interfere with the bat defense of tympanate moths (Operophtera spp.; Geometridae). *Animal Behaviour* **55**: 223-226.

VAN LANGEVELDE, F., ETTEMA, J.A., DONNERS M., WALLIS DEVRIES, M.F. & GROENEDIJK, D. 2011. Effect of spectral composition of artificial light on the attraction of moths. *Biological Conservation* **144**: 2274-2281.

VAN GRUNSVEN, R.H.A., DONNERS, M., BOEKEE, K., TICHELAAR, I., VAN GEFFEN, K.G., GROENENDIJK, D., BERENDSE, F. & VEENENDAAL, E.M. 2014. Spectral composition of light sources and insect phototaxis, with an evaluation of existing spectral response models. *Journal of Insect Conservation* **18**: 225-231.

WHITAKER, J.O. 1972 Food habits of bats from Indiana. *Canandian Journal of Zoology* **50**: 877-883.

WHITAKER, J.O., MCCRACKEN, G.F. & SIEMERS, B.M. 2009. Food habits analysis of insectivorous bats. In: Kunz, T.H. & Parsons, S. (Eds), *Ecological and Behavioral Methods for the Study of Bats*. The Johns Hopkins University Press, Baltimore., pp. 567-592.

Chapter 3

Foraging success of allotonic and syntonic bats in experimentally lit and naturally dark areas

Introduction

Successful foraging, determined by prey energy content and food intake rates, is necessary for all animals for maintenance and production (Kunz et al. 1995). Food intake rates depend on a number of abiotic (i.e. environment, weather, light) and biotic (i.e. prey-predator, competition, predation) interactions present within foraging habitats (Jenni-Eiermann & Jenni 1994, Anteau & Afton 2008). Animals are expected to focus their foraging efforts on food resources and foraging areas where they can gain the most energy at the lowest energetic cost and predation risk (MacArthur & Pianka 1966).

Successful foraging leads to energy intake and use of this energy involves the manufacture of metabolic byproducts and precursor molecules, which are transported in the blood plasma (Jenni-Eiermann & Jenni 1994). Plasma metabolites can serve as a proxy for refuelling rates by providing an indication of short-term mass changes in the individual (Jenni-Eiermann & Jenni 1994, McGuire et al. 2009a, McGuire et al. 2009b). Plasma metabolites have been used to answer ecological questions regarding foraging habitat quality (Acevedo Seaman et al. 2006, Anteau & Afton 2008, Guglielmo et al. 2005). By taking into account biotic interactions, plasma metabolites provide a representation of the quality of the foraging habitat used by animals. For example, Cerasale & Guglielmo (2010) detected higher refuelling rates in Wilson's Warblers foraging in 'lower quality' non-native habitat compared to native habitat.

They argued that the reason for this was a reduction in competitive interactions. The usefulness of plasma metabolites for accessing foraging habitat quality has been established in birds; however, plasma metabolites have also been measured in a range of other taxa (Galster & Morrison 1975, Arnould et al. 2001, McGuire et al 2009).

Bats, with their high energy turnover rates, serve as an excellent model group to assess environmental correlates in refuelling rates. Small bats undergo rapid changes in their energy stores and must forage successfully to meet high energy demands (Studier et al. 1970, Kurta et al. 1989, Kurta et al. 1990). For example, on a nightly basis captive bats may consume 10.1-21.8 % of their body mass in insect prey following intensive foraging (McGuire et al. 2009). Changes in bat refuelling rates are likewise influenced by abiotic and biotic interactions present within foraging habitats.

Many bats limit foraging to dark habitats, potentially due to lower perceived predation risk (Rydell 1992, Kuijper et al. 2008). This is particularly the case with some slower flying allotonic bats. Allotonic bats are those that have shifted the frequency of their echolocation calls to outside the hearing range of tympanate moths (these moths typically detect frequencies between 20-50 kHz; Rydell 1992). Allotonic bats therefore have access to tympanate moth prey and typically forage later in the night to reduce predation risk (Kronfeld-Schor & Dayan 2003). Human disturbances, such as artificial light, may disrupt these biotic and abiotic determinants of bat foraging behaviour.

By illuminating bat foraging habitats, artificial lighting likely alters bat foraging activity (Minnaar et al. 2015). Artificial night lighting may be altering the balance in the global arms race shared between bats and tympanate moth prey (Svensson & Rydell 1998, Svensson et

al. 2003, Minnaar et al. 2015). Artificial light appear to be causing tympanate moths to mimic diurnal behavioural strategies (when anti-bat defences would prove detrimental; Fullard et al. 2003). Moth aggregations around artificial light sources, combined with their inability to effectively evade bats (Warrant & Dacke 2011, van Grunsven et al. 2014), makes moths more accessible (Frank 1988, Rydell 1992, Svensson & Rydell 1998, Longcore & Rich 2004). High numbers of syntonic bats (those that use echolocation in the 20-50 kHz hearing range of eared moths) take advantage of artificial light by foraging nearby. In contrast, the rarer allotonic bats are often of conservation concern and seem to be declining (Rydell 1992, Acharya 1995, Svensson & Rydell 1998, Minnaar et al. 2015). A possible mechanism for this decline could be that syntonic bats have increased foraging success in areas with artificial light, relative to allotonic bats (Minnaar et al. 2015). Artificial light may attract moths from the foraging areas of allotonic bats, resulting in competition with syntonic bats. It is also possible that allotonic bats are excluded from foraging in artificially lit areas due to perceived predation risk (Polak et al. 2011, Minnaar et al. 2015).

The aim of my study was to evaluate the refuelling rates of South African allotonic and syntonic bats in experimentally lit and naturally dark areas. I will argue that this is a potential mechanism explaining the disruption of bat-prey community interactions. I measured changes in β-hydroxybutyrate (a blood metabolite), which reflects refuelling rates, to test for differences in the food intake rates of bats in artificially lit vs naturally dark areas. Plasma metabolite analysis is assumed to be a proxy for foraging success (McGuire et al. 2009a, McGuire et al. 2009b, Boyles et al. 2016), where β-hydroxybutyrate differs as a reflection of energetic state in birds and mammals (Galster & Morrison 1975, Jenni-Eiermann & Jenni 1994, Arnould et al. 2001; but see McGuire et al. 2009a). I predicted that syntonic bats would

have higher refuelling rates following foraging in artificially lit conditions (relative to natural light conditions), where they were expected to have increased foraging opportunities due to moth aggregations around lights (Rydell 1992, Minnaar et al. 2015). In contrast, I expected that allotonic bats would have higher refuelling rates when foraging in natural light conditions where natural bat-prey interactions are maintained.

Methods

To determine whether bats had higher refuelling rates in areas with artificial light than those without, I used the same experimental design described in Chapter 2 (see *Figure 2.2*) at Kleinrivier and Table Farm.

Measurement of refuelling rates:

Plasma metabolite (i.e. glycerol, triglycerides and β-hydroxybutyrate) analysis has previously been used to address ecological questions about the effects of alterations in habitat and food supply (Cerasale and Guglielmo 2010). It is based on the principle that metabolic state (fed / fasted) is reflected by circulating levels of free fatty acids transported in the blood plasma (Jenni-Eiermann and Jenni 1994; Williams et al. 1999; Guglielmo et al. 2005). During fasting, fatty acids are unbound from glycerol and transported to the liver, where they can be catabolized to ketones (i.e. β-hydroxybutyrate; BOH). In some cases, BOH replaces glucose as the primary fuel for respiration in tissues requiring a constant source of metabolic fuel (such as the brain; Robinson and Williams 1980; Ramenofsky 1990; Jenni-Eiermann and Jenni 1994; Acevedo Seaman et al. 2006; Guglielmo et al. 2005).

Plasma metabolite studies data for bats (McGuire et al. 2009a, McGuire et al. 2009b, Baloun 2015, Boyles et al. 2016) differ in comparison to those for shorebirds and passerines, which do not engage in intensive foraging flight. The data for these birds show that BOH is negatively correlated to triglyceride (TRIG) levels and interpreted to indicate mass loss (Jenni-Eiermann and Jenni 1994, Williams et al 1999, Guglielmo et al. 2005). Due to limitations in the processing rates of food while flying, aerial insectivores mobilize energy from fat stores, which increases BOH levels (as per the endurance exercise hypothesis; McGuire et al. 2009a). Elevations in BOH levels have been correlated with feeding intensity in volent insectivorous bats (McGuire et al. 2009a, McGuire et al. 2009b, Boyles et al. 2016), for which elevated ketone levels are linked to a fatty insectivourous diet (Askew et. 1975, Baloun 2015). Hence, BOH is both an indicator of short term energy gains and a fuel source during energy imbalance (McGuire et al. 2009a, McGuire et al. 2009b, Boyles et al. 2016). I elaborate on the interpretations of BOH as an energy source, originating from exogenous and/or endogenous pathways, in my discussion.

Within ten minutes of capturing an individual bat, I collected <20 – 75 µL (amounting to < 1 % of body mass, depending on the bat species) of blood by gently puncturing the brachial vein using a sterilized 27-gauge hypodermic needle (as per McGuire et al. 2009a and McGuire et al. 2009b). When necessary, I used a hot water bottle (not exceeding 35 °C) applied to the sampling site to promote blood flow. I collected blood in a heparinized capillary tube. Treatment (lit or unlit), time of capture, body mass (±0.1 g), length of the forearm (±0.1 mm) and recorded the reproductive state of each individual. To prevent duplicate sampling, I banded each bat on the forearm with a 3 mm metal ring before releasing them at the site of capture.

After collection, I centrifuged blood samples for 10 minutes at 2000 rpm using a portable centrifuge to separate plasma from blood cells. I quantified β-hydroxybutyrate concentrations with a handheld meter (STAT-Site M β-HB; Stanbio Laboratory, Boerne, TX USA) using 10 µL samples of plasma. The handheld meter is logistically feasible to measure blood plasma metabolite levels in the field (Boyles et al. 2016), as opposed to the time consuming, expensive process of assaying metabolites in a microplate spectrophotometer (Jenni-Eiermannm & Jenni 1994, Guglielmo et al. 2002, Guglielmo et al. 2005). The meter was validated using laboratory enzymatic analysis (Boyles et al. 2016, Sommers et al. 2017). All of our protocols for capturing bats and taking blood samples were approved by the Rhodes University Ethical Standards Committee (RU-DZE-2018-01-002) and Eastern Cape Department of Environmental Affairs Permit (CRO 130/17CR and CRO131/17CR).

Data analysis:

I focused analysis on two species with sufficient sample sizes: allotonic Cape horseshoe bats (hereafter referred to as allotonic bats) and syntonic dusky pipistrelles (hereafter referred to as syntonic bats). I conducted a simple regression analysis to assess the importance of variation in body mass in relation to BOH levels, for syntonic and allotonic bats respectively. I then used a regression to assess the effect of temperature on BOH levels. Prior to assessing the effect of treatment (unlit vs lit) on BOH values, I tested for normality of BOH residuals using both a Shapiro-Wilks and Levenes test. Although the assumption of homogeneity of variance was met, residual BOH values for both syntonic and allotonic bats followed a significantly non-normal distribution, requiring the use of a Kolmogorov-Smirnov test. I

subsequently tested for the effect of treatment (unlit vs lit) on BOH values using a Kruskal-Wallis test.

Results

At both sites, the majority of bats were captured in the early evening. Whereas bats at Kleinrivier were mostly trapped over water sources, bats at Table Farm were trapped along foraging flight paths 100 m from a known roost cave. Despite netting late into the night, bat activity (monitored on a Pettersson D100 ultrasound detector) declined substantially after the first two hours of darkness. This was particularly the case in summer when bats appeared to have engaged in short foraging bouts.

Body mass was not an important in predictior of BOH levels for syntonic (r2 = 0.10, p>0.05) or allotonic bats (r2 = 0.07, p>0.05). Likewise, temperature did not have an effect on BOH levels for syntonic (r2=0.40, p=0.13) or allotonic bats (r2= 0.02, p > 0.09). Within syntonic pipistrelles, a few individuals caught around lights had high BOH levels (*Figure 3.1* and *Table 3.1*), but there was no difference in BOH levels across treatments (Kruskal-Wallis $X^2_{1,27}$ = 0.89, df= 14, p=0.345). In allotonic horseshoe bats, inter-treatment BOH levels did not differ (Kruskal-Wallis $X^2_{1,33}$ = 1.83, df= 31, p=0.18; *Table 3.1, Figure 3.1*).

Table 3.1: Comparisons of β-hydroxybutyrate (BOH) levels in bats foraging in the unlit or lit treatment. Values are presented as mean ± SD. Sample sizes are given for each species (Control: Lit).

Species (n, Control: Lit)	Lit BOH (mg dL^{-1})	Unlit BOH (mg dL^{-1})	P-value
Pipistrellus hesperidus (18: 11)	11.00±12.94	6.80±7.27	0.340
Rhinolophus capensis (17:18)	5.36±2.88	3.84±3.50	0.172

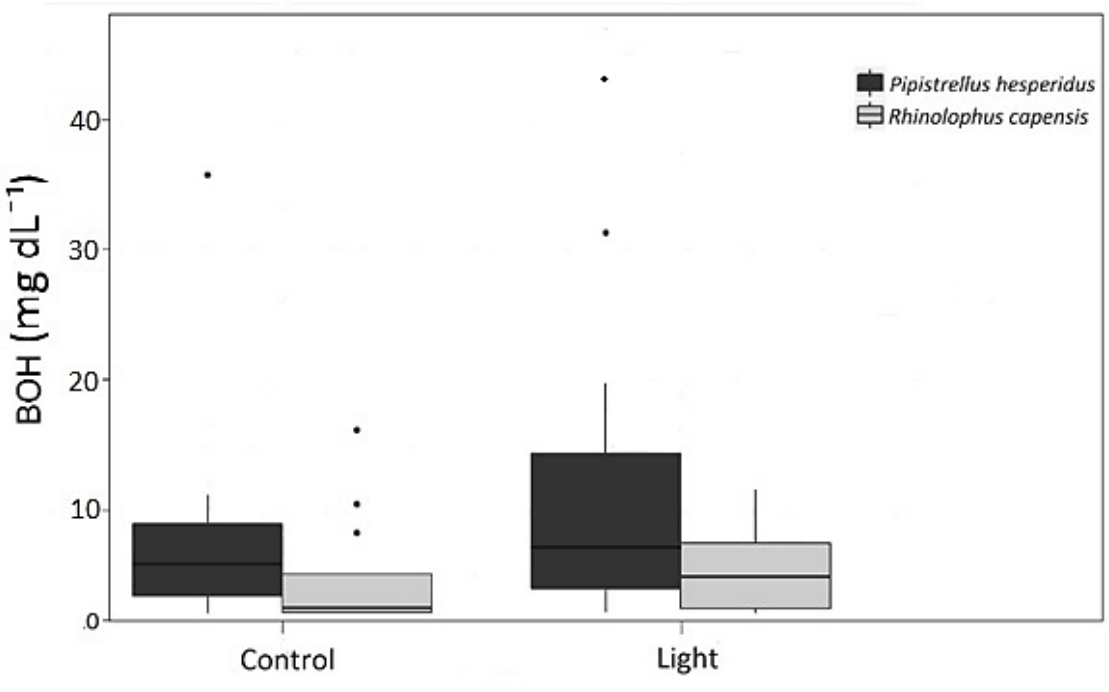


Figure 3.1: β-hydroxybutyrate (BOH) levels as a function of treatment (lit vs unlit) and bat species; BOH levels were not significantly different (p>0.05). High variance in BOH levels occurred in pipistrelles in the lit treatment, relative to the control.

Discussion

I found that ketone levels did not differ between syntonic or allotonic bats foraging in the unlit or lit treatment. These results are not consistent with my hypothesis that bat refuelling rates would differ when foraging under artificial lighting. BOH levels in dusky pipistrelles reached as high as 42 mg dL^{-1} (11.01±12.94 mg dL^{-1}; *Table 3.3*; higher than means reported in other species and attributed to the stronger metabolic effects in the small-bodied dusky pipistrelle weighing 5-6 g) in individuals foraging around the light source.

Table 3.3: Inter-treatment BOH measured in syntonic pipistrelles and allotonic horseshoe bats in relation to plasma metabolite measurements available in the literature

Metabolite	Taxon / species	Plasma metabolite value (mg dL^{-1})	Reference
B-OH	Bat: *Pipistrellus hesperidus*	Unlit: 5.30±5.16 ; Lit: 11.01±12.94 Range: 2.00-32.20; Range: 2.00-42.00	Present study (Bailey 2018)
B-OH	Bat: *Rhinolophus capenses*	Unlit: 3.84±3.50 ; Lit: 5.36±2.88 Range: 2.00-14.60 ; Range: 2.00-10.40	Present study (Bailey 2018)
B-OH	Bat: *Myotis lucifugus*	Fasted: 3.42±0.18 ; Fed: 4.86±0.18	McGuire et al. 2009a
TRIG	Bat: *Myotis lucifugus*	Fasted: 0.54±0.18 ; Fed: 7.92±1.26	McGuire et al. 2009a
TRIG	Bat: *Tadarida brasiliensis*	Maximum: 15.12 ; Maximum pregnant female: 21.60	Widmaier et al. 1996
TRIG	Shorebirds and passerines	Range: 18.00-144.00	Jenni-Eiermann and Jenni 1994; Guglielmo et al. 2002, 2005.
TRIG	Antarctic fur seal pups: *Arctocephalus gazella*	Range: 10.80-28.80	Arnould et al. 2001
TRIG	Hibernating arctic ground squirrels: *Citellus undulates*	Range: 15.30–50.94	Galster & Morrison 1975

It is inconclusive whether the higher than average BOH levels in dusky pipistrelle individuals around the light source are linked to foraging responses, suggested by the dietary shift to moth prey under lit conditions (see Chapter 2). The current knowledge of how bats burn metabolic fuel limits concrete understanding of these patterns. These individuals may have either been gaining more energy by converting food to ketones for immediate use, or spending energy stores for instantaneous flight costs to access prey resources around lights – and ultimately benefit energetically at the end of foraging bouts (McGuire et al. 2009a). Thus, the results from my short-term light exposure experiment suggest that artificial light does not affect the foraging of allotonic bats, while there is insufficient evidence to reject the null hypothesis that the foraging success of syntonic bats is equivalent in lit vs unlit conditions. As both study sites were naturally dark and a considerable distance from urban areas, an extended duration of light exposure may be required to fully habituate the syntonic bat community to foraging around artificial light.

I could not standardize the timing of trapping and numbers between treatments owing to unpredictable variations in bat activity and behaviour. A major limitation of my study was that I only caught bats early in the evening, despite netting efforts extending late into the night. Greater inter-treatment differences in refuelling rates would have been expected in bats foraging later into the evening (in agreement with the endurance exercise hypothesis; McGuire et al. 2009a, 2009b). An assumption of my study was that I captured bats at their foraging sites. Bat activity at trapping sites substantially declined following two hours after sunset, presumably as bats returned to roosts. Alternatively, bats may have moved to preferred foraging sites some distance from my netting sites, as they became accustomed to the nets. I observed shortened foraging bouts by bats at trapping sites particularly during summer. In summer, widespread insect distributions may allow for reduced dependence on aggregations around artificial light and reduced foraging times. This further limited trapping time and detectable inter-treatment foraging differences in my study.

Artificial light sources likely allow some pipistrelle species, amongst other syntonic bats (Fenton & Morris 1976, Fenton et al. 1997, Minnaar et al. 2015), easier access to tympanate moths. This is consistent with their syntonic echolocation strategy (50.1 ±1.5 kHz; Schoeman 2016) and ability to forage in fairly open habitats (Monadjem et al. 2010) characteristic of those where I deployed artificial light. Syntonic species that do well around artificial light typically use urban-adaptive echolocation, flight and foraging strategies (optimal for open urban habitats; Jones & Rydell 1994). Prior to the global increase in artificial lighting, moths were rarely reported in pipistrelle diets. Common pipistrelles (*Pipistrellus pipistrellus*) are now flourishing in urbanized environments of Switzerland where they prey on Lepidoptera and Diptera (Arlettaz et al. 2000). Simultaneous to increases in pipistrelle abundance,

decreases in sympatric Swiss populations of lesser horseshoe bats (*Rhinolophus hipposideros*) have occurred (Arlettaz et al. 2000). The homogenization of bat assemblages surrounding artificial light has been linked to dietary competition; allotonic bats may experience a direct reduction in foraging success, or indirect reduction in foraging habitat quality as moths move out of the foraging areas of allotonic bats (Arlettaz et al. 2000, Stone et al. 2009, Minnaar et al. 2015). The influx of the dusky pipistrelle (and other syntonic species) into the dietary niche of the near threatened Fynbos endemic, the Cape horseshoe bat (*Rhinolophus capensis*) should raise similar concerns regarding a compromise in the foraging efficiency and ultimately, displacement of these allotonic bats.

I did not find differences in the refuelling rates of Cape horseshoe bats between lit and unlit treatments, suggesting that this species was able to maintain sufficient access to moths as a prey resource under artificially lit conditions during my study (see Chapter 2). Following persistent efforts yielding no captures of the horseshoe bats present at Kleinrivier, horseshoe bats were sampled at Table Farm. At Table Farm, Cape horseshoe bats were captured in fynbos and karoo tree canopies which were part of a scrubland habitat, where this species is known to feed on moths and beetles (Monadjem et al. 2010). I argue that treatment differences override habitat differences at the sample sites – both sharing similar insect (see Chapter two of this study) and bat communities (Monadjem et al. 2010). While the impacts of artificial light on the foraging activity of Cape horseshoe bats was negligible, Russo et al. (2017) reported a benefit gained in another allotonic, forest-dwelling species (*Barbastella barbastellus*) foraging around artificial light. However, other allotonic species may experience visual impairment around artificial light (Hope & Bhatnagar 1979). Bat activity in lit urban areas has been negatively correlated with echolocation frequency (Schoeman 2016).

However, rather than a direct response to artificial lighting, slower flying allotonic bats may be unable to compete in artificially lit habitats, especially when these occur in open habitats (Schoeman 2016). Allotonic species may thus be more susceptible to changes in foraging activity in open lit habitats. I did not test this directly and it remains to be addressed in future studies. Artificial light may be a synergistic factor influencing reported declines in allotonic bats, but land use change (influencing loss of roosts and foraging habitat), as well as pesticide-induced declines to moth populations may have a considerable impact on allotonic bats (Arlettaz et al. 2000, Avila-Flores & Fenton 2005).

Rather than the common notion that increases in moth consumption will occur around artificial light (Acharya & Fenton 1992, 1999), other factors may also be at play. Artificial light may induce strong species-specific effects (i.e. syntonic *Myotis* spp. avoid lights; Russo et al. 2017), or have a greater impact on reducing bat drinking activity as opposed to foraging activity around productive water-bodies (detected in pipistrelle guild members; Russo et al. 2017). Furthermore, foraging benefits potentially gained in syntonic species may be short-lived, decreasing with duration of light exposure, as local abundances in pipistrelles may only occur with sufficient tree cover for roosting (Mathews et al. 2017).

In conclusion, the limitation of catching bats early in the evening may have confounded the effects of artificial lighting on foraging success, as deduced by BOH measurements. This may be prevented in future studies by using a harp trap where bats return to the roost, following foraging at experimental sites that they are accustomed to. The results from my short-term light exposure experiment suggest that artificial light does not affect the foraging of allotonic Cape horseshoe bats, while there is insufficient evidence to reject the null hypothesis that the foraging success of syntonic bats is equivalent in lit vs unlit conditions. My data emphasizes

the need for a better mechanistic understanding of the influence of artificial lighting on bat species. Bat-moth interactions may be influenced by other factors apart from the common assumption that increased refuelling rates will occur in syntonic species foraging on moths around artificial light.

References

ACEVEDO SEAMAN, D.A., GUGLIELMO, C.G., ELNER, R.W., WILLIAMS, T.D., SEAMAN, D.A.A., GUGLIELMO, C.G., ELNER, R.W., WILLIAMS, T.D. & HOBSON, K.A. 2006. Landscape-scale physiology: Site differences in refuelling rates indicated by plasma metabolite analysis in free-living migratory sandpipers. *Auk* **123**: 563-574.

ANTEAU, M.J. & AFTON, A.D. 2008. Using plasma-lipid metabolites to index changes in lipid reserves of free-living lesser scaup (*Aythya affinis*). *The Auk* **125**: 354-357.

ARLETTAZ, R., GODAT, S. & MEYER, H. 2000. Competition for food by expanding pipistrelle bat populations (*Pipistrellus pipistrellus*) might contribute to the decline of lesser horseshoe bats (*Rhinolophus hipposideros*). *Biological Conservation* **93**: 55-60.

AFNOULD, J.P.Y., GREEN, J.A. & RAWLINS, D.R. 2001. Fasting metabolism in Antarctic fur seal (*Arctocephalus gazelle*) pups. *Comparative Biochemistry and Physiology A* **129**: 829-841.

ASKEW, E.W., DOHM, G.L. & HUSTON, R.L. 1975. Fatty acid and ketone body metabolism in the rat: response to diet and exercise. *Journal of Nutrition* **105**: 1422-1432.

AVILA-FLORES, R. & FENTON, M.B. 2005. Use of spatial features by foraging insectivorous bats in a large urban landscape. *Journal of Mammalogy* **86**: 1193-1204.

BALOUN, D. 2015. The effects of recent feeding on plasma metabolites in little brown bats (*Myotis lucifugus*). Honours thesis, University of Winnipeg, Winnipeg, Canada.

BOYLES, J.G., MCGUIRE, L.P., BOYLES, E., REIMER, J.P., BROOKS, C.A.C., RUTHERFORD, R.W., RUTHERFORD, T.A., WHITAKER, J.O. & MCCRACKEN, G.F. 2016. Physiological and behavioral adaptations in bats living at high latitudes. *Physiology and Behaviour* **165**: 322-327.

BRIGHAM, R.M., & GEISER, F. 1998. Seasonal activity patterns of two species of *Nyctophilus* bats based on mist-net captures. *Australian Mammalogy* **20**: 349-352.

CERASALE, D.J. & GUGLIELMO, C.G. 2006. Plasma metabolite profiles: effect of dietary phospholipids

in a migratory passerine (*Zonotrichia leucophrys gambelii*). *Physiological and Biochemical Zoology* **79**: 754-762.

CERASALE, D.J. & GUGLIELMO, C.G. 2010. An integrative assessment of the effects of Tamarisk on stopover ecology of a long-distance migrant along the San Pedro River, Arizona. *The Auk* **127**: 636-646.

FENTON, M.B., BOYLE, N.G.H., HARRISON, T.M. & OXLEY, D.J. 1977. Activity patterns, habitat use and prey selection by South African insectivorous bats. *Biotropica* **9**: 73-85.

FENTON, M.B. & MORRIS, G.K. 1976. Opportunistic feeding by desert bats (*Myotis* spp.). *Canadian Journal of Zoology* **54**: 526-530.

FRANK, K.D. 1988. Impact of outdoor lighting on moths: an assessment. *Journal of the Lepidopterists' Society* **42**: 63-93.

FULLARD, J.H., MUMA, K.E. & DAWSON, J.W. 2003. Quantifying an anti-bat flight response by eared moths. *Canadian Journal of Zoology* **81**: 395-399.

GALSTER, W. & MORRISON, P.R. 1975. W. Gluconeogenesis in arctic ground squirrels between periods of hibernation. *American Journal of Physiology* **228**: 325-330.

GUGLIELMO, C.G., CERASALE, D.J. & ELDERMIRE, C. 2005. A field validation of plasma metabolite profiling to assess refuelling performance of migratory birds. *Physiological and Biochemical Zoology: PBZ* **78**: 116-125.

GUGLIELMO, C.G., O'HARA, P.D. & WILLIAMS, T.D. 2002. Extrinsic and intrinsic sources of variation in plasma lipid metabolites of free-living western sandpipers (*Calidris mauri*). *The Auk* **119**: 437-445.

HOPE, G.M. & BHATNAGAR, K.P. 1979. Effect of light adaptation on electrical responses of the retinas of four species of bats. *Experientia* **35**: 1191-1193.

JENNI-EIERMANNM, S. & JENNI, L. 1994. Plasma metabolite levels predict individual body-mass changes in a small long-distance migrant, the Garden Warbler. *The Auk* **111**: 888-899.

JONES, G. & RYDELL, J. 1994. Foraging strategy and predation risk as factors influencing emergence time in echolocating bats. *Philosophical Transactions of the Royal Society B* **346**: 445-455.

JUNG, K. & KALKO, E.K.V. 2010. Where forest meets urbanization: foraging plasticity of aerial insectivorous bats in an anthropogenically altered environment. *Journal of Mammalogy* **91**: 144-153.

KRONFELD-SCHOR, N. & DAYAN, T. 2003. Partitioning of time as an ecological resource. *Annual Review of Ecology, Evolution, and Systematics* **34**: 153-181.

KUIJPER, D.P.J., SCHUT, J., VAN DULLEMEN, D., TOORMAN, H., GOOSSENS, N., OUWEHAND, J. & LIMPENS, H.J.G.A. 2008. Experimental evidence of light disturbance along the commuting routes of pond bats (*Myotis dasycneme*). *Lutra* **51**: 37-49.

KUNZ, T.H., WHITAKER, J.O. & WADANOLI, M.D. 1995. Dietary energetics of the insectivorous Mexican free-tailed bat (*Tadarida brasiliensis*) during pregnancy and lactation. *Oecologia* **101**: 407–415.

KURTA, A., BELL, G.P., NAGY, K.A. & KUNZ, T.H. 1989. Energetics of pregnancy and lactation in free-ranging little brown bats (*Myotis lucifugus*). *Physiological Zoology* **62**: 804-818.

KURTA, A., KUNZ, T.H. & NAGY, K.A. 1990. Energetics and water flux of free-ranging big brown bats (*Eptesicus fuscus*) during pregnancy and lactation. Journal of Mammalogy **71**: 59-65.

LONGCORE, T. & RICH, C. 2004. Ecological light pollution. *Frontiers in Ecology and the Environment* **2**: 191-198.

MACARTHUR, R.H. & PIANKA, E.R. 1966. On optimal use of a patchy environment. *The American Naturalist* **100**: 603-609.

MATHEWS, F., ROCHE, N., AUGHNEY, T., JONES, N, DAY, J., BAKER, J. & LANGTON, S. 2015. Barriers and benefits: implications of artificial night-lighting for the distribution of common bats in Britain and Ireland. *Philosophical Transactions of the Royal Society of London, Biological Sciences*: **370**: 20140124.

MCGUIRE, L.P., FENTON, M.B., FAURE, P.A. & GUGLIELMO, C.G. 2009a. Determining feeding state and rate of mass change in insectivorous bats using plasma metabolite analysis. *Physiological and Biochemical Zoology* **82**: 812-818.

MCGUIRE, L.P., FENTON, M.B. & GUGLIELMO, C.G. 2009b. Effect of age on energy storage during prehibernation swarming in little brown bats (*Myotis lucifugus*). *Canadian Journal of Zoology* **87**: 515-519.

MINNAAR, C., BOYLES, J.G., MINNAAR, I.A., SOLE, C.L. & MCKECHNIE, A.E. 2015. Stacking the odds: Light pollution may shift the balance in an ancient predator-prey arms race. *Journal of Applied Ecology* **52**: 522-531.

MONADJEM, A., TAYLOR, P.J., COTTERILL, W., & SCHOEMAN, M.C. 2010. *Bats of southern and central Africa: a biogeographic and taxonomic synthesis*, Johannesburg: Wits University Press, pp. 596.

MUCINA, L., & RUTHERFORD, M.C. 2006. *The vegetation of South Africa, Lesotho and Swaziland*. South African National Biodiversity Institute.

NORBERG, U.M., & RAYNER, J.M.V. 1987. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Philosophical Transactions of the Royal Society of London, B. Biological Sciences* **316**: 335-427.

POLAK, T., KORINE, C., YAIR, S. & HOLDERIED, M.W. 2011. Differential effects of artificial lighting on flight and foraging behaviour of two sympatric bat species in a desert. *Journal of Zoology* **285**:21-27.

RAMENOFSKY, M. 1990. Fat storage and fat metabolism in relation to migration. In: Gwinner, E. (Ed), *Bird migration*. Springer-Verlag, Berlin, pp. 214-231.

ROBINSON, A.M. & WILLIAMSON, D.H. 1980. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiological Reviews* **60**: 143-187.

RUSSO, D., CISTRONE, L., LIBRALATO, N., KORINE, C., JONES, G., & ANCILLOTTO, L. 2017. Adverse effects of artificial illumination on bat drinking activity. *Animal Conservation* **20**: 492-501.

RYDELL, J. 1992. Exploitation of insects around streetlamps by bats in Sweden. *Functional Ecology* **6**: 744-750.

SCHOEMAN, M.C. 2016. Light pollution at stadiums favors urban exploiter bats. *Animal Conservation* **19**: 120-130.

SCHOEMAN, M.C. & JACOBS, D.S. 2003. Support for the allotonic frequency hypothesis in an insectivorous bat community. *Oecologia* **134**: 154-162.

SOMMERS, A.S., Boyle, W.A., & MCGUIRE, L.P. 2017. Validation of a field-ready handheld meter for plasma β-hydroxybutyrate analysis. *Journal of Field Ornithology*.

STONE, E.L., JONES, G. & HARRIS, S. 2009. Street lighting disturbs commuting bats. *Current Biology* **19**: 1123-1127.

STUDIER E.H., PROCTOR, J.W. & HOWELL, D.J. 1970. Diurnal body weight loss and tolerance of weight loss in five species of *Myotis*. *Journal of Mammalogy* **51**: 302-309.

SVENSSON, A.M., EKLOF, J., SKALS, N., RYDELL, J. & SVENSSON, A.M. 2003. Light dependent shift in the anti-predator response of a pyralid moth. *Oikos* **101**: 239-246.

SVENSSON, A.M. & RYDELL, J. 1998. Mercury vapour lamps interfere with the bat defense of tympanate moths (Operophtera spp.; Geometridae). *Animal Behaviour* **55**: 223-226.

VAN GRUNSVEN, R.H.A., DONNERS, M., BOEKEE, K., TICHELAAR, I., VAN GEFFEN, K.G., GROENENDIJK,

D., BERENDSE, F. & VEENENDAAL, E.M. 2014. Spectral composition of light sources and insect phototaxis, with an evaluation of existing spectral response models. *Journal of Insect Conservation* **18**: 225-231.

WARRANT, E. & DACKE, M. 2011. Vision and visual navigation in nocturnal insects. *Annual Review of Entomology* **56**: 239-254.

WILLIAMS, T.D., GUGLIELMO, C.G., EGELER, O. & MARTYNIUK, C.J. 1999. Plasma lipid metabolites provide information on mass change over several days in captive western sandpipers. *Auk* **116**: 994-100.

ZAJAC, R.M., CERASALE, D.J., GUGLIELMO, C.G., ZAJAC, R.M., CERASALE, D.J. & GUGLIELMO, C.G. 2017. The rapid response of plasma metabolites to changes in feeding rate in a small passerine Wilson's Warbler *Wilsonia pusilla*. *Journal of Avian Biology* **37**: 405-408.

Chapter 4

Main findings and conclusion

Through the role of bats as first-order predators, bat community structure is an excellent indicator of habitat quality (Ghanem & Voigt 2012). However, bat community structure may be compromised by artificial lighting through a reduction in the defences of tympanate moths, thereby increasing competition for moth prey (Svensson & Rydell 1998, Schoeman 2016). Several studies have reported the monopolizing of insect aggregations around artificial lights by bats (Rydell 1992, Minnaar et al. 2015, Schoeman 2016), some making use of dietary shifts towards moth prey (Minnaar et al. 2015). Research on the effect of artificial light on bat foraging ecology has focused on moth specialists (Acharya & Fenton 1992, Acharya & Fenton 1999), leading to the largely untested paradigm that increases in moth consumption will occur around lights. However, the hypothesized increase in moth consumption by bats around lights cannot be applied universally across all species and communities.

While artificial lighting reduces the importance of echolocation in determining the dietary presence of moth prey, my findings demonstrate that this will not result in increased moth consumption by all syntonic bat species. Syntonic bats may increase selection for moth prey around lights, but this does not appear to occur in syntonic species naturally consuming a high proportion of non-eared moths, or those specializing on beetle prey (which I assessed qualitatively). In contrast, I found that syntonic bats exhibiting generalist foraging strategies (such as *Pipistrellus* and *Tadarida* species which are common in urban areas; Arlettaz et al. 2000, Avila-Flores & Fenton 2005, Schoeman 2016) elevated moth consumption around lights. Small bodied pipistrelles are likely to benefit most from foraging on soft-bodied moth

prey (as opposed to sclerotized beetle prey requiring greater digestion efforts). *Pipistrellus hesperidus* was more selective for moth prey in lit conditions. However, it is inconclusive whether the higher than average BOH levels, in some individuals captured around the light source, are linked to foraging responses. There is insufficient evidence to reject the null hypothesis that the foraging success of syntonic bats is equivalent in lit vs unlit conditions. The high BOH levels in some syntonic individuals around light alludes to other potential influential factors. For example, artificial light has been shown to have a greater impact on bat drinking activity, relative to foraging activity, with the duration of light exposure being an important factor (Russo et al. 2017).

In my study, allotonic bats exhibited no variation in foraging activity or diet around lights, where impacts on moth populations sufficient to influence allotonic species may only be evident with prolonged light exposure. Cape horseshoe bats (*Rhinolophus capensis*) are unlikely to be drastically impacted even with prolonged light exposure, as this species consumes both moth and nutrient-rich beetle prey based on seasonal variability (Monadjem et al. 2010). Allotonic species unable to make use of diet shifts, limited either by alternative prey availability or suitable skull morphology, may be impacted by reductions in moth prey occurring with prolonged light exposure. Given differences in community responses to artificial light, a greater mechanistic understanding of bat-prey interactions is required to determine the susceptibility of bat species to changes in foraging ecology (Cravens et al. 2017).

To prevent the homogenization of bat assemblages (Schoeman 2016) and maintain trophic generalist interactions imposed by generalist foraging syntonic bats (Fenton 1977), it is

necessary to plan for sustainable urban development. Natural bat and insect activity can be maintained by placing filters over lights to block UV emissions (Frank 1988) or using longer-wavelength artificial light (van Grunsen et al. 2014). As opposed to white or green light, long wavelength red light allows light-sensitive bat species and generalist syntonic species to forage as they would in natural darkness (Spoelstra et al. 2017). However, human use of broad spectrum lighting is more likely to drive the development of lighting technology, rather than conservation (Davies et al. 2013, Minnaar et al. 2015). Alternatively, artificial lighting can be reduced spatially and temporally to limit ecological impacts. Biologists, land-owners and city planners should be encouraged to enact legislation enforcing the motion sensor lighting technology of walk-way, street and warehouse lighting – in this way reducing the carbon footprint, urban sky glow and financial costs (Gaston et al. 2013, Kyba et al. 2014, Minnaar et al. 2015). Reserves with associated dispersal corridors should be maintained as light free refuges for light-sensitive species to maximize the benefits of artificial lighting while reducing the ecological impacts on bat-prey interactions.

References

ACHARYA, L. & FENTON, M.B., 1992. Echolocation behaviour of vespertilionid bats (*Lasiurus cinereus* and *Lasiurus borealis*) attacking airborne targets including arctiid moths. *Canadian Journal of Zoology* **70**: 1292-1298.

ACHARYA, L. & FENTON, M.B., 1999. Bat attacks and moth defensive behaviour around streetlights. *Canadian Journal of Zoology* **77**: 27-33.

ARLETTAZ, R., GODAT, S. & MEYER, H. 2000. Competition for food by expanding pipistrelle bat populations (*Pipistrellus pipistrellus*) might contribute to the decline of lesser horseshoe bats (*Rhinolophus hipposideros*). *Biological Conservation* **93**: 55-60.

AVILA-FLORES, R. & FENTON, M.B. 2005. Use of spatial features by foraging insectivorous bats in a large urban landscape. *Journal of Mammalogy* **86**: 1193-1204.

CRAVENS, Z.M., BROWN, V.A., DIVOLL, T.J. & BOYLES, J.G., 2017. Illuminating prey selection in an insectivorous bat community, exposed to artificial light at night. *Journal of Applied Ecology*.

DAVIES, T.W., BENNIE, J., INGER, R., DE IBARRA, N.H. & GASTON, K.J. 2013. Artificial light pollution: Are shifting spectral signatures changing the balance of species interactions? *Global Change Biology* **19**: 1417-1423.

FRANK, K.D. 1988. Impact of outdoor lighting on moths: an assessment. *Journal of the Lepidopterists' Society* **42**: 63-93.

GASTON, K.J., BENNIE, J., DAVIES, T.W., HOPKINS, J. 2013. The ecological impacts of nighttime light pollution: A mechanistic appraisal. *Biological Reviews* **88**: 912-927.

GHANEM, S.J. & VOIGHT, C.C. 2012. Increasing awareness of ecosystem services provided by bats. *Advances in the Study of Behavior* **44**: 279-302.

KYBA, C.C.M., HANEL, A. & HOLKER, F. 2014. Redefining efficiency for outdoor lighting. *Energy and Environmental Science* **7**: 1806-1809.

MINNAAR, C., BOYLES, J.G., MINNAAR, I.A., SOLE, C.L. & MCKECHNIE, A.E. 2015. Stacking the odds: Light pollution may shift the balance in an ancient predator-prey arms race. *Journal of Applied Ecology* **52**: 522-531.

MONADJEM, A., TAYLOR, P.J., COTTERILL, W., & SCHOEMAN, M.C. 2010. *Bats of southern and central Africa: a biogeographic and taxonomic synthesis*, Johannesburg: Wits University Press, pp. 596.

RUSSO, D., CISTRONE, L., LIBRALATO, N., KORINE, C., JONES, G., & ANCILLOTTO, L. 2017. Adverse effects of artificial illumination on bat drinking activity. *Animal Conservation* **20**: 492-501.

RYDELL, J. 1992. Exploitation of insects around streetlamps by bats in Sweden. *Functional Ecology* **6**: 744-750.

SCHOEMAN, M.C. 2016. Light pollution at stadiums favors urban exploiter bats. *Animal Conservation* **19**: 120-130.

SPOELSTRA, K., VAM GRUNSVEN, R.H.A, RAMAKERS, J.C., FERGUSON¸ K.B., RAAP. T., DONNERS, M., VEENENDAAL, E.M. & VISSER, M.E. 2017. Response of bats to light with different spectra: light-shy and agile bat presence is affected by white and green, but not red light. *Proceedings of the Royal Society B: Biological Sciences* **284**: 20170075.

SVENSSON, A.M. & RYDELL, J. 1998. Mercury vapour lamps interfere with the bat defense of tympanate moths (Operophtera spp.; Geometridae). *Animal Behaviour* **55**: 223-226.

VAN GRUNSVEN, R.H.A., DONNERS, M., BOEKEE, K., TICHELAAR, I., VAN GEFFEN, K.G., GROENENDIJK,

D., BERENDSE, F. & VEENENDAAL, E.M. 2014. Spectral composition of light sources and insect phototaxis, with an evaluation of existing spectral response models. *Journal of Insect Conservation* **18**: 225-231.

Supplementary information

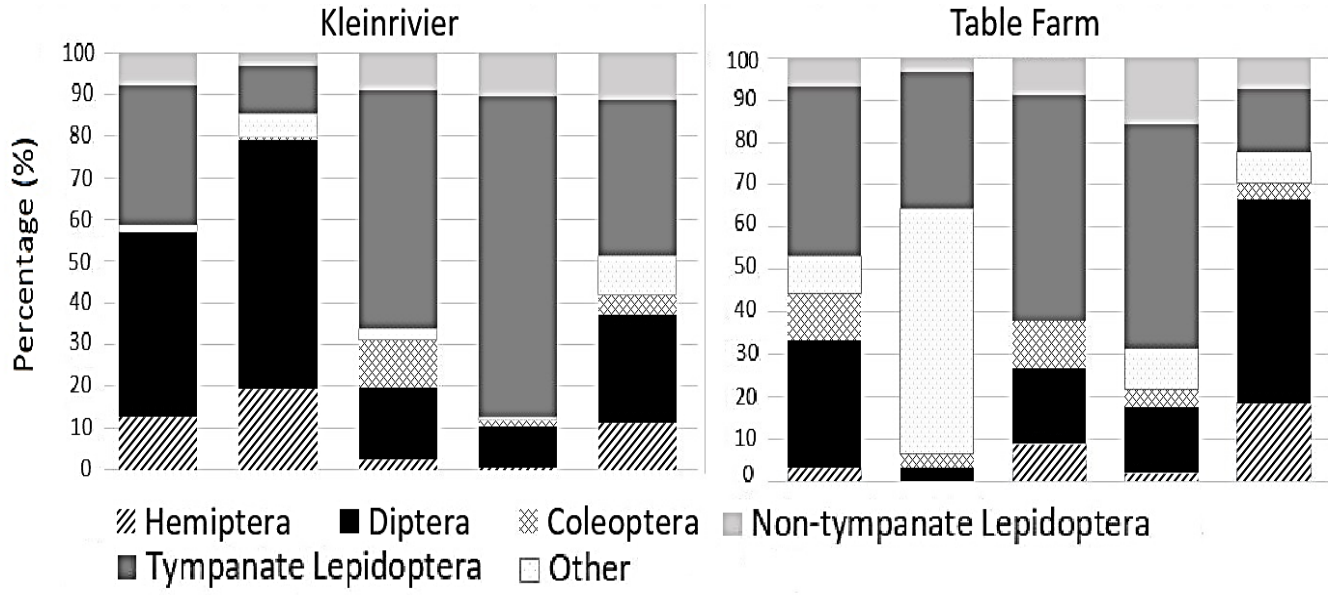
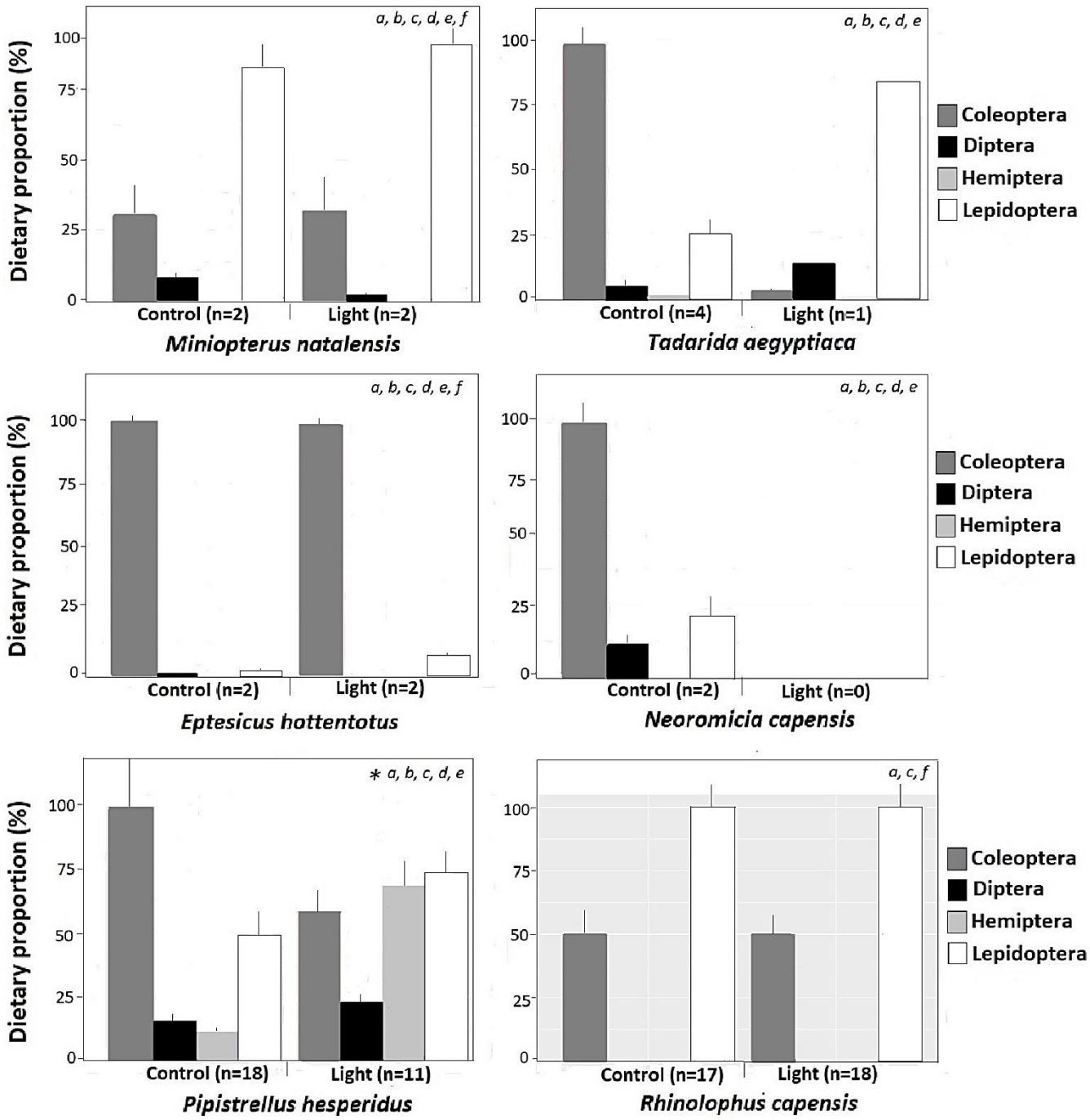


Figure S1: The proportion of insect comprising the prey community potentially available to bats Insect communities at Kleinrivier (Site 1) and Table Farm (Site 2). Bars represent timing of sampling intervals.



Supplementary S2: Variation in the proportion of insect orders consumed by syntonic (white panel) and allotonic (shaded panel) species across lit and unlit treatments.
Dietary proportions are reflective of percentage volume dietary constituents. Letters (top right of each panel) are indicative of dietary differences between bat species (for all insect orders), where those bat species that do not share a letter had significantly different diets. A significant difference in intraspecies moth consumption between treatments is indicated by an asterix; *P. hesperidus* exhibited significant increases in moth consumption under artificial lights (*).

Table S1: The proportion (%) of eared and non-eared moths constituting the total sampled prey community at Kleinrivier (Site 1) and Table Farm (Site 2).

	Site 1	Site 2
Eared moths:		
Pyralidae	30.59	10.91
Geometridae	9.77	3.22
Noctuidae	11.19	14.00
Notodontidae	7.19	6.45
Sphingidae*	0.59	3.23
Non-eared moths:		
Cossidae	5.74	2.96
Crambidae	3.80	-
Tineidae	8.00	6.11
Yponomeutidae	1.06	-
Pieridae	0.93	-
Limacodidae	-	1.96
Thyretidae	2.22	-